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TENTH ANNIVERSARY YEAR
1956–57

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ANNUAL REPORT

An Outline of Research during the Fiscal Year 1956-1957

BULLETIN NO. 36 · JANUARY 1958

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B. S. SCHWEIGERT · *Director of Research and Education*

AMERICAN MEAT INSTITUTE FOUNDATION
939 EAST 57TH STREET · CHICAGO 37 · ILLINOIS

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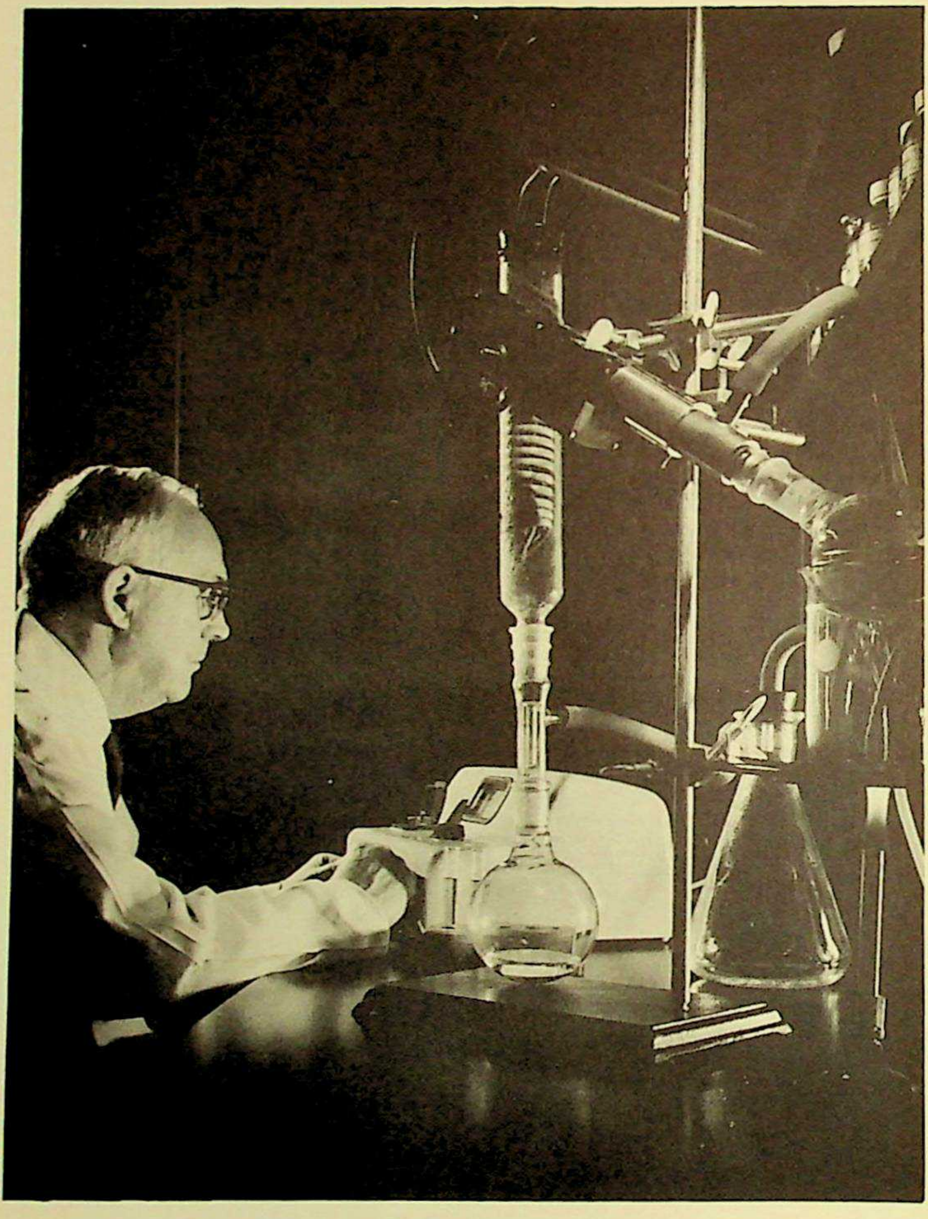

The chemistry of proteins separated from cattle hides is probed by Dr. Frank L. DeBeukelaer, who retired recently as Chief of the Division of Hides and Skins.

American Meat Institute Foundation

Organization. The American Meat Institute Foundation is a non-profit organization affiliated with and located at the University of Chicago. It is the outgrowth of an all-industry research program initiated by the American Meat Institute in 1924 and maintained by that organization for more than two decades. Valuable scientific contributions were made in the processing and utilization of livestock products by the Research Laboratory of the Institute. This work demonstrated the practicality of industry group research and led the Institute to make a survey of the needs for an expanded research program and possible methods of achieving such expansion. In 1944 the Institute's Board of Directors, acting on the recommendation of the Institute staff, voted to sponsor the establishment of an independent, non-profit organization to carry out a program of research and education in livestock production, processing, and utilization. The Foundation was incorporated in 1944 and began operations on a moderate scale in 1947. In 1949 the Foundation moved into a new laboratory building on the campus of the University of Chicago. This structure is designed to meet the specific needs of the Foundation and, with equipment, represents an investment of more than a million dollars. The funds for construction and equipment of the building were contributed by several hundred meat-packing companies—ranging from the smallest to the largest—and by companies in allied industries.

Purpose. The Foundation was organized to carry out a broad and comprehensive program of research and education in the field of livestock production and processing, including the utilization of livestock products. In the latter connection, technological advances resulting from intensive research in other industries have already induced major displacements of important livestock by-products. Thus, if the livestock, meat, and by-products industries are to keep abreast of the advancing times, improved or more economical processing methods, new and improved products, and new uses for such products must be developed. Such important advancements, of course, can result only from consistent and expanding attention to the types of fundamental research and technological experimentation now being conducted at the Foundation.

Affiliation with the University of Chicago. The Foundation's location in the heart of one of the great research centers of the country and its affiliation with the University of Chicago provide distinct advantages. There is opportunity for exchange of scientific information between the Foundation staff and scientists of the University, as well as with scientists with other research organizations on the campus. Some Foundation staff members hold professorial appointments in the University. A number of graduate students are doing thesis work in the Foundation's laboratories under the supervision of these Foundation scientists.

An Advisory Committee, three members of which are appointed by the University and two by the Foundation, serves to co-ordinate the programs of the two institutions in such a manner as to obtain the maximum benefit to the public and to the co-operating institutions.

A Development Committee, with three members from the University and two from the Foundation, holds as trustees, for the benefit of the public and the Foundation, any patents that may result from any invention or discovery in research financed by the Foundation.

Financial Support. The Foundation was established, and in large degree is maintained, by voluntary financial support from the livestock and meat industry and others interested in the development of scientific information that will lead to improved utilization of livestock.

Such contributions are deductible by donors for income tax purposes, since the Foundation is a non-profit organization and all income is used exclusively for scientific and educational purposes. Any bequests, legacies, devices, or transfers similarly are deductible in arriving at the value of the net estate of a decedent for estate tax purposes.

Provision has been made for the Foundation to render service in research for industry, the Department of Agriculture, the Department of Defense, and other governmental agencies. The Foundation conducts research for outside agencies in fields in which it can function most effectively because of its personnel, facilities, and experience. Grants and contributions for special research projects are accepted. Gifts and donations likewise are accepted in support of the purposes of the Foundation.

Research planning begins with careful study of all subject information previously developed. Quick reference Thermofax reproduction of pertinent data here is prepared by Barbara A. Heisler for AMIF scientist.

Technical Staff of the Foundation

*H. R. KRAYBILL, Ph.D., Vice-President and Director of Research and Education
B. S. SCHWEIGERT, Ph.D., Director of Research and Education and Chief of the Division of Biochemistry and Nutrition
D. M. DOTY, Ph.D., Associate Director of Research and Education and Chief of the Division of Analytical and Physical Chemistry
C. F. NIVEN, JR., Ph.D., Associate Director of Research and Education
EARL AUERBACH, Ph.D., Assistant to the Director
H. A. ARMSTRONG, Chief of the Division of Information and Service
+F. L. DeBEUKELAER, Ph.D., Chief of the Division of Hide Research
L. R. DUGAN, JR., Ph.D., Chief of the Division of Organic Chemistry
J. B. EVANS, Ph.D., Chief of the Division of Bacteriology
J. W. GIFFEE, Ph.D., Chief of the Division of Hide Research
H. WANG, Ph.D., Chief of the Division of Histology
C. EDITH WEIR, Ph.D., Chief of the Division of Home Economics
O. H. M. WILDER, Ph.D., Chief of the Division of Animal Feeds
G. D. WILSON, Ph.D., Chief of the Division of Food Technology
J. E. MARONEY, B.S., Chief of the Service Laboratory
L. J. PIRCON, M.S., Chemical Engineer
R. H. DEIBEL, M.S., Associate Bacteriologist
J. B. FOX, JR., Ph.D., Associate Biochemist
C. H. LUSHBOUGH, Ph.D., Associate Biochemist
E. P. MARBACH, Ph.D., Associate Chemist
W. R. MARTIN, Ph.D., Associate Bacteriologist
A. J. SIEDLER, M.S., Associate Biochemist
R. A. SLIWINSKI, Ph.D., Associate Chemist
M. C. URBIN, Ph.D., Associate Food Technologist
L. A. WITTING, Ph.D., Associate Biochemist
O. F. BATZER, B.S., Assistant Chemist
MARION L. BIRKNER, M.S., Assistant Histologist
W. R. CHESBRO, B.S., Assistant Bacteriologist
SUZANNE D. DRAKE, M.S., Assistant Bacteriologist
BARBARA R. GREGORY, B.S., Assistant Biochemist
P. A. LOQUERCIO, B.S.Ch.E., Assistant Engineer
FRANKLIN MILLS, M.A., Assistant Food Technologist
J. POBUTSKY, Ch.E., Assistant Chemist
G. SAKELLARIDES, B.S., Assistant Chemist
H. T. SLOVER, B.S., Assistant Chemist
JO ANN JOHNSON, M.S.L.S., Librarian
DORIS M. MORELAND, B.S., Bookkeeper
J. M. BARON, Assistant
C. BERNOFSKY, B.S., Assistant
P. D. BROWN, B.S., Assistant
LUCY D. CHANG, B.S., Assistant

---

* Deceased, September 30, 1956.
+ Retired, September 28, 1956.

B. J. CHAO, B.S., Assistant
MELICENT R. CHUTKOW, B.S., Assistant
MARION P. CULLEN, M.S., Assistant
PATRICIA A. DAVIDSON, Assistant
AUDREY D. FREEMAN, B.S., Assistant
ELIZABETH B. GINGER, B.A., Assistant
D. J. GINSBERG, B.A., Assistant
A. V. JENSEN, M.S., Assistant
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R. S. BERK, M.S., Graduate Research Assistant
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D. E. LAKE, B.S., Graduate Research Assistant
S. W. MOLINE, B.S., Graduate Research Assistant
F. A. NAYDER, Graduate Research Assistant
E. C. STELLWAGEN, B.S., Graduate Research Assistant
T. C. FLAX, Sampler
H. G. SCHILLER, Sampler

GRADUATE STUDENTS

Othmer F. Batzer	Division of Biochemistry and Nutrition
Richard S. Berk	Division of Bacteriology
William R. Chesbro	Division of Bacteriology
Robert H. Deibel	Division of Bacteriology
Charles R. Heisler	Division of Biochemistry and Nutrition
Donald E. Lake	Division of Bacteriology
Sheldon W. Moline	Division of Biochemistry and Nutrition
Francis A. Nayder	Division of Biochemistry and Nutrition
Arthur J. Siedler	Division of Biochemistry and Nutrition
Earle C. Stellwagen	Division of Biochemistry and Nutrition

FELLOWS

Woodrow J. Aunan, F. C. Vibrans Senior Scientist Fellow (1957)
Richard S. Berk, W. Lee Lewis Fellow
Martti Knuuttila, Fulbright Fellow from Finland
I. S. Schneider, Research Fellow from Brazil
Sheldon W. Moline, H. R. Kraybill Fellow

GRADUATE STUDENTS GRANTED DEGREES

From The University of Chicago

Charles R. Heisler, Doctor of Philosophy, 1957, Department of Biochemistry
Donald E. Lake, Master of Science, 1956, Department of Microbiology.

Activities and Accomplishments
Tenth Anniversary Year
1956-1957

The American Meat Institute Foundation has completed, during the fiscal year 1956-1957, ten years of active research in broad fields designed to provide important information relating to the preservation and processing and utilization of meat and meat products. These have been years of growth and accomplishment—years during which we have built a highly effective scientific staff; have devised and projected a research program of singular breadth and significance; and have established a distinguished record for scientific capacity, resourcefulness, and achievement.

The Foundation and its research and educational program and activities and its accomplishments are now well known throughout the world of science and education and in the meat-packing and allied industries, both on this continent and abroad. Forty-eight publications on the results of diversified research in many fields of interest have been issued this year and are tabulated elsewhere in this report. AMIF publications now total more than three hundred, and these scientific and technical reports and general publications are in constant demand, with requests coming not only from all parts of the United States and Canada but from many countries in Europe, Asia, and South America. Companies in several of the countries abroad are represented in the list of those participating in the financial support of the Foundation's research program.

In consequence of its affiliation with the University of Chicago and its well-established record of scientific accomplishment and integrity, the Foundation has had the privilege of sharing in the educational advancement of a steadily widening circle of young scientists. Eight University of Chicago students have obtained Ph.D. degrees with Foundation staff members, and twelve have received M.S. degrees. Several of these graduates have been retained on the Foundation staff, some are affiliated with food companies, and others are now on the teaching and research staffs of universities and land-grant colleges throughout the United States. Nine graduate students carried out research in the Foundation's laboratories during this report year. One student received the Ph.D. degree in biochemistry and a second received the degree of Master of Science in microbiology.

Four fellowships have been established this year under authority provided by the Foundation's Board of Directors. Two fellowships were established in honor of the late Drs. W. Lee Lewis, former Director of Research for the American Meat Institute, and Henry R. Kraybill, first Director of Research and Education of the Foundation, and are for students working with AMIF staff members toward advanced degrees at the University of Chicago. A third fellowship honors the memory of Dr. Frank G. Vibrans, noted research leader in the field of fat and oil technology and for many years a member of the staffs of both the Department of Scientific Research of the American Meat Institute and the Foundation. The Vibrans fellowship provides for residence studies at the Foundation during the summer months for an outstanding research and teaching scientist affiliated with some university or college in the United States. The fourth fellowship has been awarded in support of a graduate student working toward a Ph.D. degree at the University of Florida.

By direction of the Foundation's Board of Directors, a bronze plaque has been struck to recognize the successful efforts, in the early 1920's, of five industry and educational leaders to stimulate collaborative support of scientific and technological research relating to the processing and utilization of meat and other products derived from livestock. The Foundation is a progressive outgrowth of the pioneering laboratories created under the leadership of Mr. Thomas E. Wilson, then head of Wilson and Company, Inc.; Mr. Oscar G. Mayer, head of Oscar Mayer and Company and also then President of the American Meat Institute; Mr. Emery T. Filbey, now Vice-President Emeritus of the University of Chicago and then Dean of the University College; and the late Messrs. Arthur Lowenstein, then Director of Research for Wilson and Company, Inc.; and William Whitfield Woods, then Vice-President and executive head of the American Meat Institute. The plaque has been placed in the lobby of the Foundation laboratories.

The outstanding research accomplishments of the Foundation during its ten years of research activity were highlighted during the Fifty-first Annual Meeting of the American Meat Institute in a paper presented by Dr. Bernard S. Schweigert, AMIF Director of Research and Education, and by an extensive exhibit prepared especially for the meeting.

As we complete our tenth anniversary year, expanded attention is being given to many new areas of long-range research, including the water-holding capacity of meats, proteolytic enzymes, nutritional studies on fats and atherosclerosis, rancidity in pork products, oxidation of fats by microorganisms, and the chemical nature of collagen proteins.

It may be noted that, during the last year:

Grants-in-aid were received from the National Live Stock and Meat Board, The United States Public Health Service, Merck and Company, Inc., the National Institutes of Health, and the United States Atomic Energy Commission.

Contracts in support of several research projects were received from the Quartermaster Food and Container Institute for the Armed Forces and the United States Department of Agriculture.

One or more members of the staff attended meetings of the American Chemical Society, American Feed Manufacturers Association, Federated Biological Societies, Society of American Bacteriologists, National Renderers Association, Institute of Food Technologists, Reciprocal Meat Conference, American Home Economics Association, American Leather Chemists' Association, American Association of Anatomists, and other societies. Approximately forty-eight papers were presented by the staff during this past year at these meetings and others.

The Service Laboratory analyzed 8,476 samples from 300 companies during this past year.

Dr. J. B. Evans was promoted to Chief of the Division of Bacteriology, and Dr. J. Walter Giffee was appointed Chief of Hide Research. Other persons joining the staff during the year were Lucy Chang, M. Saeed, Franklin Mills, G. D. Maier, M. R. Chutkow, M. P. Cullen, A. V. Jensen, N. M. Risser, and I. S. Schneider (Fellowship).

Research Projects 1956-1957

Meat Tenderization

Basic Research on Substances or Agents with Potential Meat-tenderizing Properties

(H. Wang, C. Edith Weir, Marion L. Birkner, and Betty Ginger)

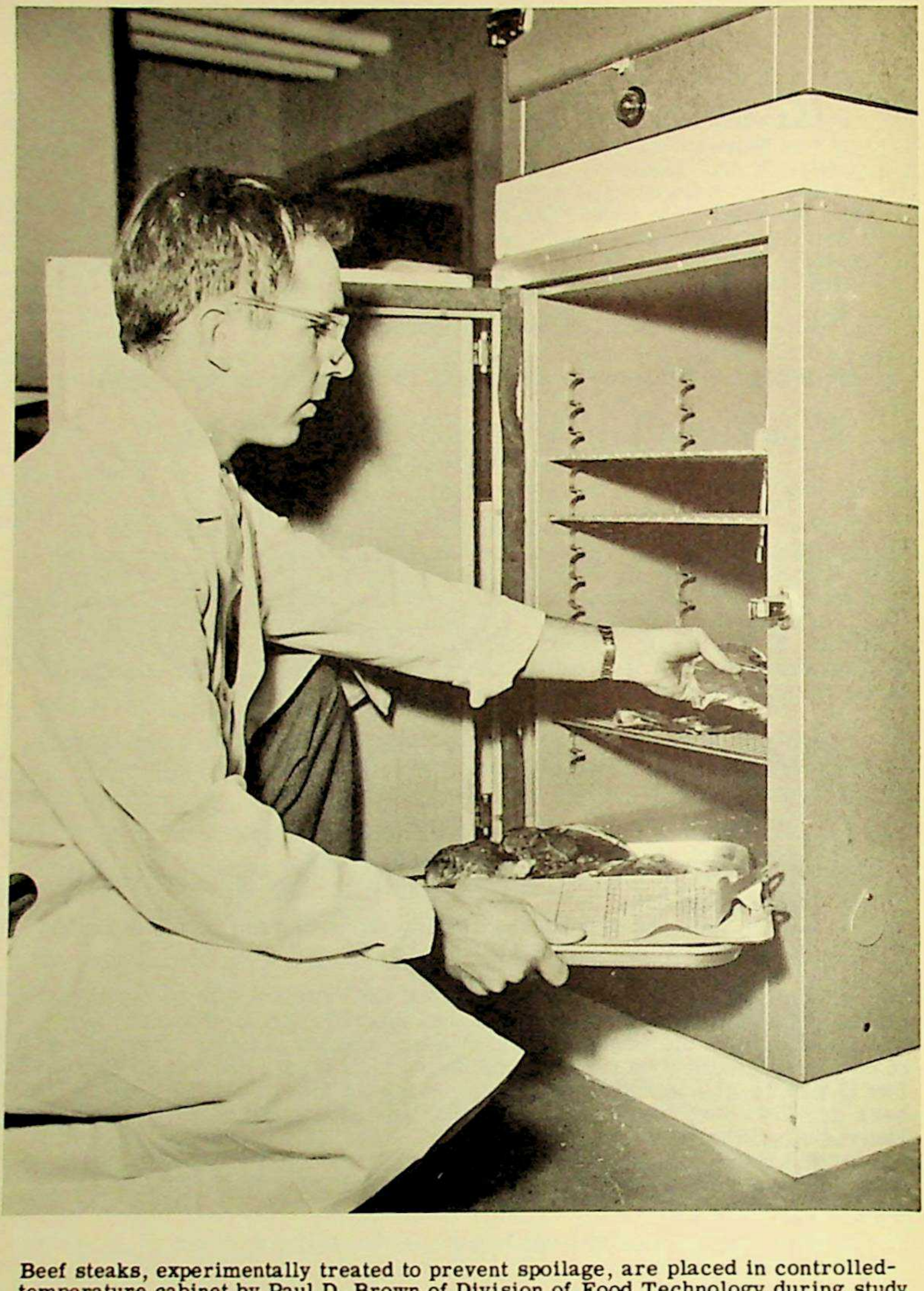
(Supported in part by a grant from Hercules Powder
Company and Paul-Lewis Laboratories)

Taste-panel scoring, shear-force measurements, muscle-fiber diameter, and extensibility measurements were employed in a study of the rate of action of Age-It, a commercial tenderizer. The rate of tenderization on 3/4-inch steaks cut from utility grade Longissimus dorsi muscles was more rapid during the first 2 hours after treatment than during the following 16 hours. On a similar, but initially less tender, muscle, a measurable increase in tenderness was noted 15 minutes after treatment of the steaks and overtenderization 2 hours after treatment.

A study of individual tenderizing agents was undertaken. Preliminary investigation of the effect of inorganic ions upon meat tenderization included:

1. The extent of penetration of NaCl and NH^4Cl into fresh meat
2. The effect of cooking upon this penetration
3. The effect of ions upon muscle-fiber diameter and extensibility measurements
    a) On isolated muscle fibers directly immersed in salt solutions
    b) On steaks treated superficially with salt solutions

In the case of isolated muscle fibers, the extent of change in fiber diameter and extensibility varied with the specification used (Na, K, Mg, and Ca) and with the concentration of each solution. An approximate line of demarkation may be drawn between the high (7, 10, and 14 per cent) and low (2 per cent) concentrations of the salt solutions used. The muscle fibers greatly increased in both diameter and extensibility in the high concentrations, whereas, in the low concentration, only slight swelling of the immersed fibers occurred, accompanied by a decrease in extensibility. Similar results were noted in solutions of 2 per cent NaCl, KCl, and $MgCl^2$, but not $CaCl^2$.



Beef steaks, experimentally treated to prevent spoilage, are placed in controlled-temperature cabinet by Paul D. Brown of Division of Food Technology during study of tenderization by high-temperature aging.

Studies on High-Temperature Aging To Increase Tenderness of Lower Grades of Beef

(P. D. Brown, W. R. Chesbro, Betty A. Ginger,
C. Edith Weir, G. D. Wilson, C. F. Niven, Jr.)

(Supported in part by contract with the
United States Department of Agriculture)

Various organic acids, antibiotics, and gamma irradiation, singly and in combination, have been evaluated as preservatives during the aging of beef at elevated temperatures.

Initially, intact whole rounds and top rounds were used. Steaks were removed serially during the aging period. This method did not provide sufficient randomization to compensate for the variability between animal, sides, and location. The design of the experiment was then changed to steaks cut from the rounds, prior to aging.

A modified Latin Square design was used to compare the tenderizing temperatures of 35°, 60°, 90°, and 110° F. Antibiotic infusion satisfactorily controlled bacterial spoilage at 60° and 110° F. but was ineffective at 90° F. External spoilage during aging was satisfactorily controlled by anaerobic packaging.

Preliminary experiments, using antibiotics in combination with gamma irradiation ($1\text{-}2 \times 10^5$ rep), indicated that microbial spoilage can be controlled at 90° F. Studies are being continued to determine the most practical level of irradiation to be used in connection with antibiotics.

Improving the Quality of Freeze-dried Steaks through Application of Proteolytic Enzymes

(H. Wang, C. Edith Weir, Marion L. Birkner, and Betty Ginger)

(Supported in part by contract with the Quartermaster Food
and Container Institute for the Armed Forces)

1. Histological manifestations of collagenase activity of a series of enzymes were studied by utilizing the tissue component isolated from freeze-dried steaks. Microbial enzymes appeared to exert their effect only on the ground substance or matrix of the connective tissue. However, a more extensive sequence of changes occurred when raw collagen was subjected to the action of ficin, bromelin, or papain. Earliest among these was a liberation of the ground substance, then a decrease in staining capacity, followed by a loss of both staining capacity and structure of the fibers, resulting finally in an amorphous material.

2. Tenderness effects of varying concentrations of five enzymes (amylase, bromelin, rhozyme, ficin, and papain), applied singly to freeze-dehydrated Longissimus dorsi steaks, were studied organoleptically. The

addition of 2 per cent sodium chloride to the rehydrated solution produced a tenderizing effect additive to that of the enzyme. The increase in tenderness produced by the 2 per cent salt was of sufficient magnitude that it is recommended that this alone be used when rehydrating Longissimus dorsi steaks.

Steaks rehydrated in 2 per cent salt resorbed more water and lost less during cooking than those rehydrated in water alone. The enzymes, at the concentrations employed, did not appreciably affect either the water resorption or the cooking loss.

3. The same series of enzymes was applied to freeze-dehydrated Semitendinosus steaks obtained from the round. Here the increase in tenderness was of more practical significance than was the increase shown for Longissimus dorsi steaks and is reflected in two distinct properties:

a) The first effect was upon the initial impression of tenderness (as previously noted with Longissimus dorsi).
b) On some occasions the initial impression of tenderness was complicated by a large amount of residual material after a short period of chewing.

The amount of this residue was reduced by bromelin, ficin, and papain. Amylase and rhozyme had no, or slight, effect upon this property when used at acceptable levels.

This residue effect can be related to the histological effects of these enzymes. The three enzymes having the ability to reduce the amount of residue also act upon the connective tissue of the meat—the others were shown not to have this histological manifestation.

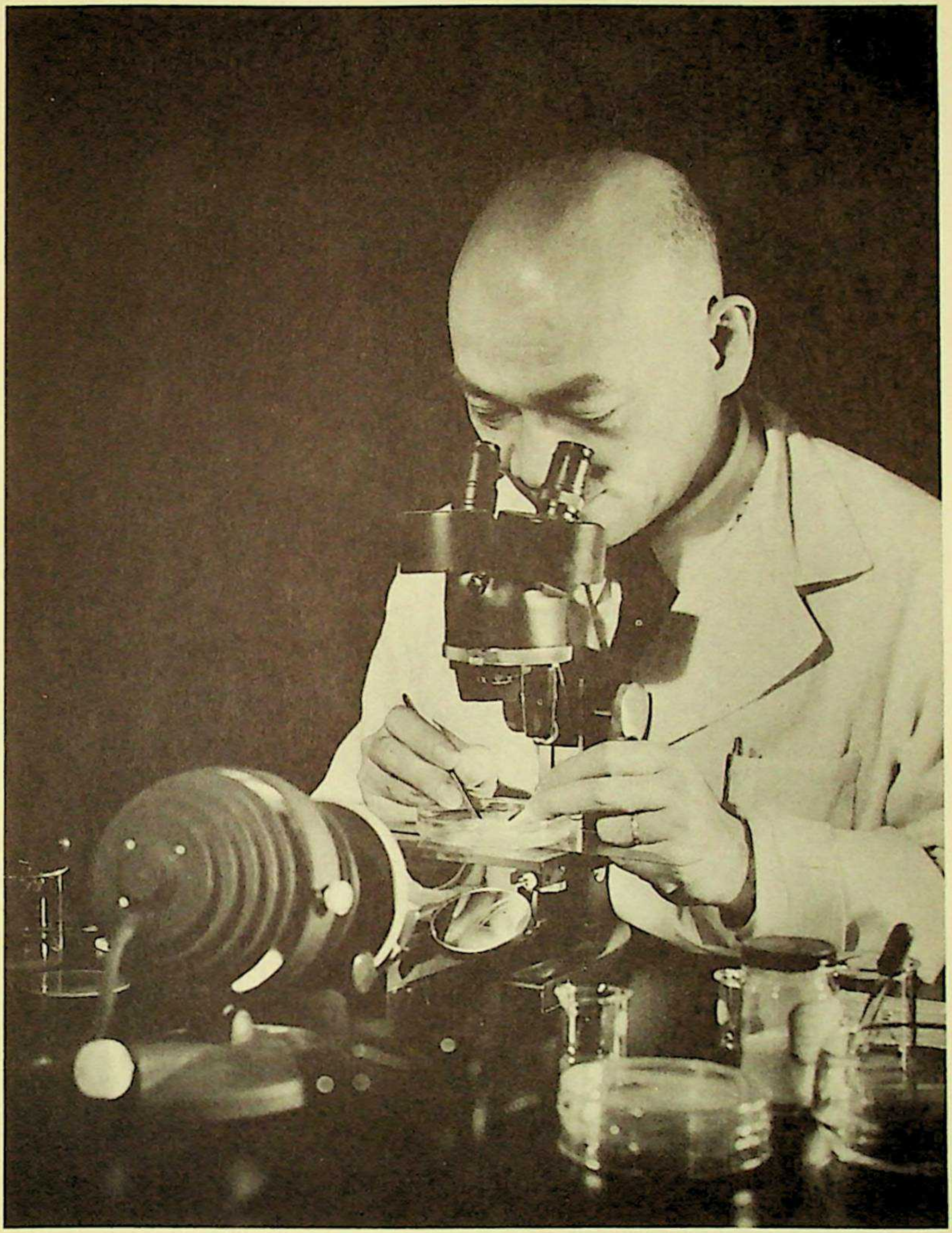
4. The above series of enzymes, in combinations with each other and at varying concentrations, were tested organoleptically. The combinations all increased tenderness and decreased residue, but in varying degrees. Individual animal differences in response to this method of tenderizing are such that a survey including many animals would be needed in order to arrive at suitable concentrations for recommendation.

Effect of Proteolytic Enzyme Treatment on the Flavor, Texture, and Rehydration Characteristics of Precooked Freeze-dehydrated Meat

(H. Wang, C. Edith Weir, Marion Birkner, and Betty Ginger)

(Supported in part by contract with the Quartermaster
Food and Container Institute for the Armed Forces)

General quality observations have been made on steaks precooked, by several methods, prior to freeze-drying. Past experience at the AMIF and Quartermaster Food and Container Institute formed the basis for arriving at the precooking procedures to be used in this study. Preliminary work, involving histological examination of raw- and cooked-meat connective tissues exposed to enzyme solutions at room temperature, suggest that the changes brought about in the cooked fibers are less extensive than those occurring in the raw.



Dr. Harry Wang, Chief of Histology, makes microscopic examination of effect of proteolytic enzyme treatment on physical structure of beef during tenderization studies at Foundation.

Freeze-dried steaks are rehydrated in proteolytic enzyme solutions as Betty Ann Ginger, of Home Economics, prepares meat for cooking and taste-panel evaluation of tenderization achieved by enzyme treatment.

Nutrition

Studies on the Function of Vitamin B_{12} in Nucleic Acid Metabolism

(Francis Nayder, Othmer Batzer, A. J. Siedler,
and B. S. Schweigert)

(Supported by grants from the Division of Biology and
Medicine, Atomic Energy Commission, and the
United States Public Health Service)

The nutritional requirements of Lactobacillus acidophilus R-26, an organism which has a specific requirement for deoxyribosides which is not replaceable by vitamin B_{12}, have been studied previously.

The relative activities of a number of deoxyribonucleoside and deoxyribonucleotide compounds were determined. The activities of the deoxyribonucleotides were generally lower than those of the deoxyriboside compounds.

The activity of the deoxyribonucleotides was found to be dependent on the presence or absence of ribonucleotides in the medium. In the absence of ribonucleotides, the activity of the deoxyribonucleotides was approximately equivalent to thymidine. Deoxyguanylic acid, however, was poorly utilized in all cases.

The inhibition of the utilization of deoxyribonucleotides also varies with the specific ribonucleotide present and is reversible. Phosphate present in the medium at concentrations up to 0.1 M did not significantly influence the responses observed.

Thymidylic acid utilization is inhibited by adenylic and guanylic acid but is not significantly inhibited by cytidylic acid. Deoxycytidylic acid utilization is inhibited by adenylic, guanylic, and cytidylic acid.

Detailed studies of this inhibition phenomenon are currently being done in order to determine the inhibition indexes of the various ribotides in the presence of deoxyadenylate, thymidylate, and deoxycytidylate.

The relationship of vitamin B_{12} to nucleic acid metabolism has been investigated in L. leichmannii 313 (ATCC 7830), one of the organisms commonly used for B_{12} assays. In this organism there is a double requirement for B_{12} replacement. This double requirement consists of a source of active purine and a source of chemically combined deoxyribose. Guanine, hypoxanthine, and xanthine are active as purine sources. Adenine is inactive as a source of total purine. Cyosine deoxyriboside, thymine deoxyriboside, adenine deoxyriboside, and guanine deoxyriboside are equally active as a source of deoxyribose. Compounds such as guanine deoxyriboside and hypoxanthine deoxyriboside, which contain both an active purine and deoxyribose, can replace B_{12} alone. The B_{12} concentration required for purine synthesis appears to be twenty times that required for deoxyribose synthesis. Either the free base or the riboside of 4-amino, 5-

imidazole carboxamide is inactive as a source of purine. This work has permitted the postulation of two sites of action for B_{12} in purine synthesis: the formylation of glycinamide ribotide and/or the formylation of 4-amino, 5-imidazole carboxamide ribotide.

Previous experimental evidence using L. leichmannii indicated vitamin B_{12} functions in the conversion of ribose to deoxyribose. Totally labeled carbon 14 cytidine was injected into rats which were on B_{12}-supplemented or B_{12}-deficient diets. The mononucleotides were separated, concentrated, and purified by ion-exchange chromatography, and their radioactivity was determined. Comparisons were made of the radioactivity of nucleotides from B_{12}-supplemented and B_{12}-deficient animals.

A Re-evaluation of the Vitamin B_6 Content of Meats

(Jean F. Murray, C. H. Lushbough, and B. S. Schweigert)

(Supported in part by a grant from the
National Live Stock and Meat Board)

Microbiological yeast assay and rat bioassay methods were employed for the determination of vitamin B_6, and fresh and cooked samples of beef, lamb, veal, and pork, and several processed-meat products were assayed for vitamin B_6 content.

The results obtained in these studies indicated that previously reported values for the retention of vitamin B_6 in meats after cooking or heat-processing are too low (20-35 per cent). Values in the range from 45 to 65 per cent retention of vitamin B_6 in cooked meats have been observed repeatedly in these studies, and good agreement in the retention values was obtained by means of both the microbiological yeast assay and the rat bioassay.

The values observed for the vitamin B_6 content of the fresh-meat studies were approximately the same as those previously reported, using the microbiological yeast assay. Values obtained using the rat bioassay were approximately twice as great, both for the fresh and for the cooked and processed meats, as those observed with the yeast assay.

These results indicate that meat and meat products may provide a substantially greater proportion of vitamin B_6 in the diet than was thought previously. Recent studies relating vitamin B_6 deficiencies to infant convulsions and anemias and sensory neuritis in man emphasize the importance of this study on the re-evaluation of meats as a major source of this vitamin.

The Relation of the Type and Amount of Fat and Protein in the Diet to Heart Disease

(C. H. Lushbough, L. A. Witting, S. W. Moline,
and B. S. Schweigert)

Male weanling rats were fed experimental rations containing increasing levels of protein, at each of two levels of either of two chosen fats included in the diet. For purposes of comparison, several groups were fed rations in which carbohydrate was substituted isocalorically for fat. Such substitutions resulted in lower rates of gain and reduced caloric efficiency of feed utilization to support growth, when either low or high levels of protein were fed.

Samples of blood were taken from each animal after 6 months, and studies of the blood serum levels of cholesterol and polyunsaturated fatty acids are presently being initiated. Further blood samples will be taken at later dates; and at the conclusion of the experiment examinations of the heart and associated blood vessels will be made for evidence of the development and degree of severity of atherosclerosis.

Utilization of Lysine, Methionine, and Tryptophan from Meat Subjected to Varying Degrees of Heat Treatment

(C. H. Lushbough, Melicent R. Chutkow,
C. Edith Weir, and B. S. Schweigert)

(Supported in part by a grant from the
National Live Stock and Meat Board)

Previous studies on the quantitative utilization of lysine, methionine, and tryptophan from meats and other foods suggested that cooking or heat-processing may result in reduced availability of each of these essential amino acids. The present study was undertaken to determine the effect of heating meats for various periods of time at chosen temperatures on the availability of lysine, methionine, and tryptophan to support normal body function of the experimental animal (growth of the male weanling rat).

These experiments are being conducted by the methods employed in the earlier studies, with further technical refinements. Since this study has just been initiated, results are not yet available.

Role of Folic Acid and Related Factors in Metabolism

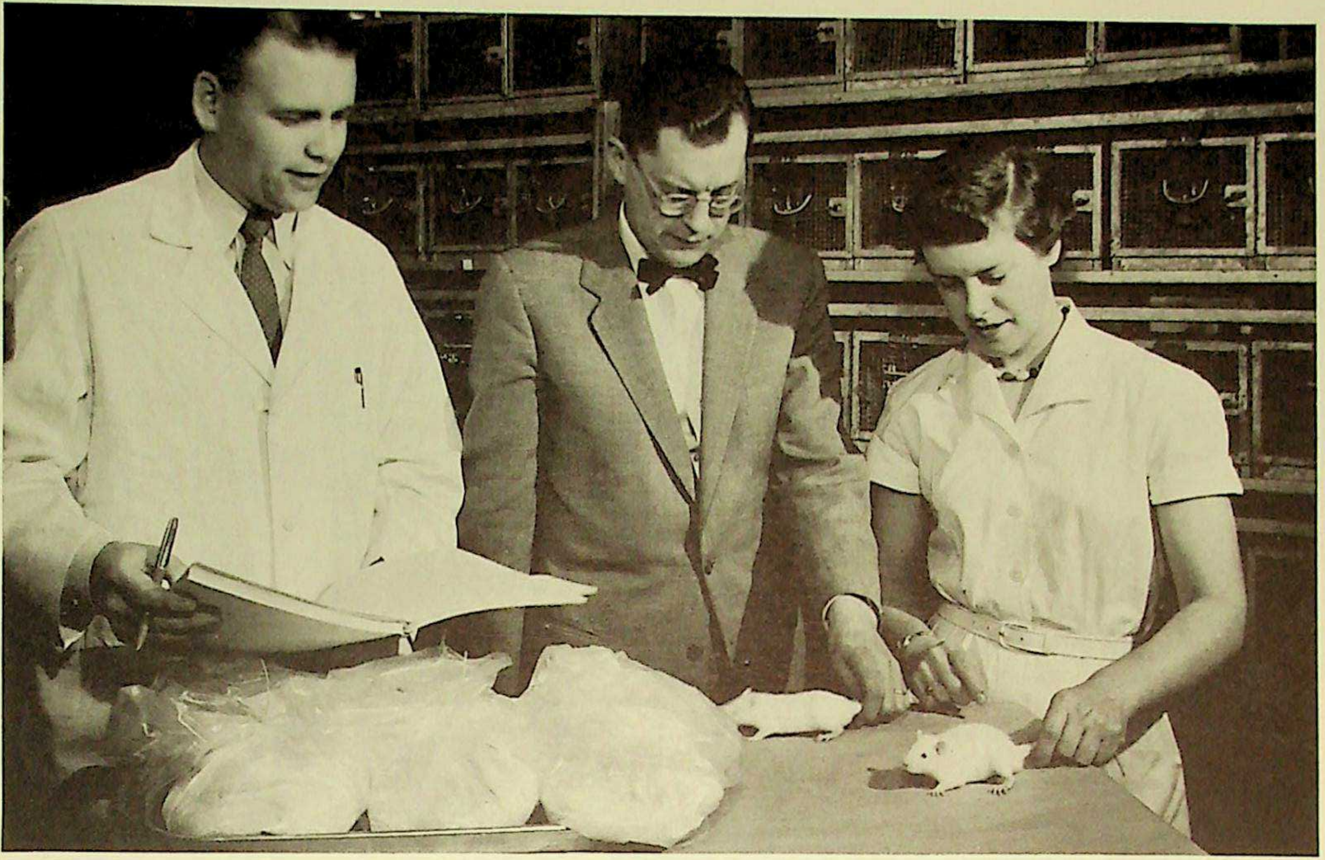
(C. R. Heisler, Earl Stellwagen, and B. S. Schweigert)

(Supported in part by a grant from the United States
Public Health Service)

Studies have been continued to determine how the B vitamin, folic acid, is converted to a reduced, formylated derivative (termed the "citrovorum factor") by enzyme systems from bacteria. The citrovorum factor appears to be the form of the vitamin that functions in the metabolism of amino acids and other vital constituents of the living cell.

We have studied the nature and amounts of all constituents necessary to bring about this conversion rapidly. The constituents required include certain B vitamin derivatives, phosphate, the amino acid serine, manganese ions, and an unidentified constituent that appears to be related chemically to folic acid itself.

Optimal conversion of folic acid to the citrovorum factor occurred in 0.06 M phosphate at pH 6.3 and at an incubation temperature of 25^{o}-26^{o} C.



Dr. B. S. Schweigert, AMIF Director of Research (center), and Dr. C. H. Lushbough and Jean F. Murray, of Biochemistry and Nutrition, record growth rates of laboratory rats during vitamin B_6 studies.

Triphosphopyridine nucleotide and ascorbic acid were required for optimal conversion in this enzyme system. Adenosine triphosphate and reduced diphosphopyridine nucleotide appeared to have a slight stimulating effect with all enzyme preparations.

The studies conducted thus far indicate that the unidentified constituent required for rapid formation of the citrovorum factor is a triglutamyl derivative of the citrovorum factor.

Nutrition of the Micro-organisms Associated with Greenish Discoloration on Sausages

(R. H. Deibel, J. B. Evans, and C. F. Niven, Jr.)

The current phase of this project has been confined to the development of a microbiological assay for thiamin, using the greening lactobacillus, *Lactobacillus viridéscens*.

Thiamin is one of the few vitamins for which analytical laboratories generally employ a chemical, rather than a microbiological, method for quantitative assay. A large number of micro-organisms have a nutritional requirement for thiamin, and several of them have been proposed for the microbiological determination of this vitamin. However, each of these proposed methods suffers from one or more disadvantages—such as lack of specificity, interference by inhibitory substances, non-specific stimulatory substances, or complexity of medium and procedure.

As we have reported previously, *L. viridescens* has an unusually high requirement for thiamin and achieves little or no growth in APT broth prepared with certain lots of yeast extract. This thiamin-deficient APT broth has proved to be an excellent basal medium for thiamin assay with *L. viridescens* as the test organism. Experimental lots of this medium have been prepared in dehydrated form by Difco Laboratories (Bacto-LV Thiamine Assay Medium), who expect to make it available commercially.

This method is convenient, rapid, relatively specific, and less subject to interfering substances as compared to previously described methods for determination of this vitamin. Cocarboxylase has about 60 per cent of the activity of equimolar quantities of thiamin. The pyrimidine and thiazole moieties of thiamin are not utilized, either individually or together, and the results are not affected by reasonable levels of pentoses, fructose, maltose, calcium, thioglycollate, or acetate.

The Effects of Amino Acid Imbalances on Micro-organisms

(W. R. Chesbro and J. B. Evans)

Glycine, when included in the growth medium in concentrations greater than 0.05 M, inhibited yeasts, bacilli, and lactic acid bacteria. In addition, it produced marked morphological changes in some of these organisms.

The effect of fourteen different amino acids on various strains of enterococci has also been studied in a standard growth medium at different pH values. The enterococci can initiate growth in this medium at pH 10.4-10.6. The toxicity of serine at neutral pH values appeared to have some taxonomic value. At pH values above neutrality, the toxicity of the amino acids was greatly increased. Comparative studies on the toxicity of various peptides of some of these amino acids are under way at the present time.

The unique ability of these enterococci to initiate growth in the test medium at pH values above 10.0 offered the possibility of studying the assimilation of amino acids into the intracellular pool above their second pK values. Colorimetric and chromatographic methods were adapted and devised for this study. Thus far, the assimilation of aspartic acid, alanine, and tyrosine by a strain of Streptococcus faecium has been studied. The preliminary evidence indicates that, although glucose fermentation proceeds above pH 10.0, none of these amino acids is assimilated above pH 9.8. All of them are assimilated at this pH, even though it is above the second pK of tyrosine. Above the third pK of tyrosine, fermentation is inhibited, and the cellular amino acid pool is liberated.

Nutritional Studies on the Minute Streptococci

(W. R. Martin and C. F. Niven, Jr.)

(Supported in part by a grant from the
National Institutes of Health)

Studies have continued concerning the metabolic roles of oleic acid and CO_2 in the metabolism of growing minute streptococci. Nutritional studies have indicated that under growth conditions oleic acid does not function in purine and pyrimidine metabolism, nor does it appear to influence the amount of CO_2 assimilated or the end products of this CO_2 fixation. It was also demonstrated that media, without oleate, exposed to 10 per cent CO_2 in air for as little as 1 hour would support the growth of these organisms when inoculated after this exposure period. It has also been shown that dissolved CO_2 is required throughout the growth cycle, not merely to get out of the lag phase.

Washed cells, resuspended in a complete growth medium, were able to fix radioactive CO_2. All the radioactivity was confined to the cellular components, no significant amount being secreted into the suspending medium. This was in contrast to cells grown under radioactive CO_2, which secreted approximately 50 per cent of the fixed CO_2 into the medium.

The presence of oleate in the suspending medium increased two- to threefold the amount of CO_2 fixed in resting cells within 1 hour. Separation and identification of the compounds containing the bulk of radioactivity in these cells are under way.

Interrelationships in the Metabolism of Tryptophan and Nicotinic Acid

(Sheldon Moline* and B. S. Schweigert)

Evidence that the enzymatic oxidation of 3-hydroxyanthranilic acid quantitatively formed quinolinic acid and that an intermediate compound accumulated during this ring-scission, ring-closure was reported earlier. The object of the present study was to elaborate a sensitive tool to study further the mechanism of formation of quinolinic acid and nicotinic acid. It becomes apparent that the nature of the reactions involving the ring carbon atom at the number 3 position of 3-hydroxyanthranilic acid was of paramount importance during the rearrangement to quinolinic acid and a nine-step chemical synthesis from o-nitroaniline-1-C^{14} was designed to prepare 3-hydroxyanthranilic acid exclusively labeled in the number 3 ring position.

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*H. R. Kraybill Fellow, 1957.

Preliminary experiments using non-labeled o-nitroaniline have shown the feasibility of the proposed synthesis. Further experiments are being continued to obtain 3-hydroxyanthranilic acid prior to usage of the labeled starting compound. An over-all yield of 10-15 per cent is expected.

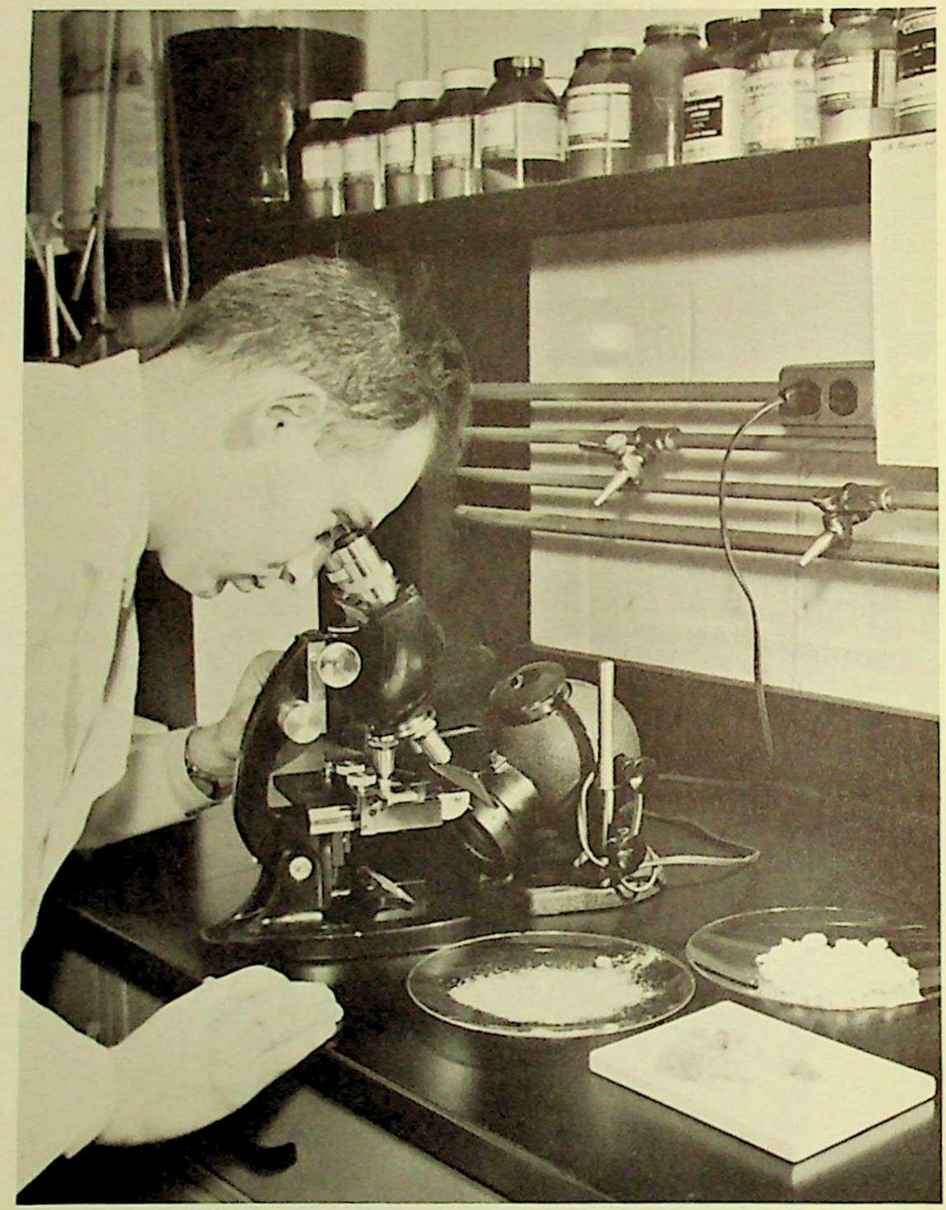
Techniques for measuring substrate disappearance, product formation, decarboxylation of quinolinic acid, and the determination of nicotinic acid have been developed with unlabeled 3-hydroxyanthranilic acid for use with experiments containing the labeled compounds.

Polyalcohol Metabolism by Lactic Acid Bacteria

(R. H. Deibel and J. B. Evans)

The fermentation of mannitol by certain strains of Streptococcus faecium is stimulated by sodium pyruvate, and considerable amounts of CO_2 are produced under these conditions. Mannitol is fermented by some strains of Lactobacillus plantarum (a homofermentative species) when added pyruvate is present, but fermentation does not occur in the presence of other hydrogen acceptors, such as fumarate.

AMIF bacteriologists have determined that sausage-greening microörganisms are efficient in a newly developed microbiological assay for the vitamin, thiamin. Here R. H. Deibel centrifuges cultures in preparation for an assay for thiamin.



Graduate Student Sheldon Moline studies intermediate products accumulated during studies designed to synthesize the manner in which the human body converts the amino acid, tryptophan, into the vitamin, niacin. Moline was the recipient of the AMIF H. R. Kraybill Fellowship for 1957.

Radiation Processing

The Radiation Sensitivity of Economically Important Meat-Spoilage Micro-organisms

(Suzanne DeWees Drake, Richard Berk, James B. Evans,
and C. F. Niven, Jr.)

(Supported in part by contract with the Quartermaster
Food and Container Institute for the Armed Forces)

Studies have continued on the effect of irradiation on the shelf-life of various meat products. Fresh beef, wrapped in cellophane, irradiated with 100,000 rep, and stored at 30°-35° F. in a humid atmosphere, retained a satisfactory appearance up to 12 days, while unirradiated controls became brown after 8 days. The application of 10 p.p.m. of chlortetracycline also extended the shelf-life to 12 days, and the combination of antibiotic and irradiation gave a 16-19-day shelf-life, as judged visually.

Samples of fresh pork loin, irradiated with 150,000 rep, had little, or no, increase in shelf-life at 30°-35° F. After 2 weeks' storage there was no significant difference in microbial count between samples, although irradiated samples were apparently free of Gram-negative rods and had more yeasts than did non-irradiated samples.

Irradiation of frankfurters with 250,000 rep did not extend their shelf-life significantly unless the microbial population before irradiation was very low. This, and even lower, levels of irradiation had a slightly adverse effect on the color of the franks.

Extensive studies on the irradiation of canned hams, before and after heat-processing, have been initiated. Results on this phase are too limited to be meaningful at the present time.

Heat-resistance and radiation-resistance studies have been conducted on enterococci that have been encountered frequently as spoilage organisms in canned hams. Their radiation resistance ranged from 153,000 to 682,000 rep (dose required to kill 99 per cent). Streptococcus faecium strains were generally more resistant than S. durans and S. faecalis strains.

Some of the strains of S. faecium were also relatively heat-resistant for non-spore-forming bacteria. Most S. faecium strains required 1-2 hours at 145° F. or 30-60 minutes at 150° F. for complete sterilization, whereas S. faecalis strains were killed by 1 hour at 145° F. or 15 minutes at 150° F. Of some interest was the fact that the three most heat-resistant strains of S. faecium were isolated from canned hams and the three least heat-resistant strains of this species were isolated from the gut of pigs. There was no apparent correlation between the heat resistance and radiation resistance of these strains.

Chemistry of Flavor and Odor Changes in Irradiated Beef

(O. F. Batzer, R. A. Sliwinski, Lucy Chang, and D. M. Doty)

(Supported in part by contract with the Quartermaster
Food and Container Institute for the Armed Forces)

Co-ordinated experiments were conducted to determine the effect of several variables on the chemical changes in irradiated meat. The variables used were: fat content, pre- and postirradiation storage time and temperature, and irradiation dosage.

For these experiments, paired loins and ribs from three grades of beef—Choice, Good, and Utility—were used, which produced the necessary range in fat content. The rib eye and its continuation in the loin were ground and subjected to 0, 2, 4, and 8 x 10^6 rep irradiation dosages. A portion was analyzed immediately, and the other portion was stored for 3 months at 30°, 60°, and 90° C. The other set was cut in sections and aged for 3 weeks at 0°, 35°, and 45° C. At the end of this time they were handled in the same manner as the first set. All samples were analyzed for changes in glutathione content, carbonyl formation, hydrogen sulfide and mercaptan formation, glycogen content, and changes in pH. Changes, such as color, odor, and texture, were also recorded.

Although the results have not as yet been exhaustively analyzed, several definite trends have been noted. These include a decrease in glutathione on irradiation and an increase in storage and aging at higher temperatures—an increase in carbonyl compounds on irradiation but a decrease during storage. The same was observed for hydrogen sulfide and mercaptan. Glycogen is decreased during irradiation and storage, and pH increases in irradiation, aging, and storage. Taste-panel evaluation of the fresh and irradiated (2 x 10^6 rep) meat in two separate experiments generally did not agree. While these and other trends have been noted, the data will have to undergo statistical analyses in order to ascertain the significance of these effects.

Some work has been done in attempts to identify the type and possible source of the acid-soluble carbonyl compounds obtained from irradiated meat. Results indicate that the bulk of the 2,4-dinitrophenylhydrazone derivatives obtained are dicarbonyl in nature, with approximately 8 per cent unsaturated or alpha keto compounds. No detectable quantities of saturated carbonyls were observed. Since the derivatives behaved like sugar derivatives in their solubility and with paper chromatography, glycogen and chondroitin sulfate were investigated as possible sources. Irradiated solutions of glycogen gave several derivatives which behaved similarly to those obtained from irradiated meat when chromatographed. When glycogen was added to meat, there was no increase in carbonyl compound. Chondroitin sulfate also produced a derivative to either an unsaturated or alpha keto derivative obtained from meat. However, when these compounds were added to meat at the 1.0 per cent level prior to irradiation, no significant increase in carbonyl compounds was observed after irradiation. Lecithin, casein hydrolyzate, glutathione, and hemoglobin, when added at the 1.0 per cent level, also did not increase carbonyl compounds on irradiation.

Chemistry of Color Changes That Occur during Irradiation of Meat

(J. B. Fox, Jr., C. Bernofsky, and B. S. Schweigert)

(Supported in part by contract with the Quartermaster
Food and Container Institute of the Armed Forces)

It was known from earlier work that the irradiation of myoglobin in meat extracts resulted in the formation of two pigments, one of which was red and therefore acceptable, and one of which was green, which was the principal cause of discoloration. This investigation was undertaken to determine the chemical characteristics of these two pigments. If oxygen is kept away during irradiation of meat, no serious discoloration is observed.

The most important factor in meat or meat extracts for the production of the green heme compound was the pH, the total amount of this pigment produced being greatest at pH 5.3. The pH of fresh meat ranges about this figure, and the "greening" can be eliminated by raising the pH to neutrality.

It has been found that irradiating either dialyzed or highly purified solutions of myoglobin at sterilizing dosages resulted in complete loss of pigment. This behavior implies that there are dialyzable compounds which exert a protective effect on the pigment, but attempts to isolate such compounds from dialyzates of muscle extracts have been unsuccessful. Experiments involving the addition of various reagents to dialyzed muscle extracts in an effort to duplicate the observed crude-extract effect have met with no success either, but it was found that the addition of sulfhydryl reagents greatly enhanced the production of the green compound. If the irradiation-induced breakdown of these sulfhydryl reagents is arrested, as by the addition of aldehydes, it is found that the amount of green pigment is decreased. This evidence, in addition to a study of the chemical behavior of the green compound after it is produced, identifies the pigment as sulfmyoglobin, formed during irradiation by the reaction of myoglobin with hydrogen peroxide and hydrosulfide from the breakdown of sulfhydryl reagents already present in meat.

Cleavage of the green pigment yields but one iron-porphyrin complex, identified chromatographically and spectrally as acid hematin, indicating that the heme moiety of the compound has not been altered. On the other hand, physical studies of the pigment show that the globin moiety has been extensively altered. The green compound is unstable in solution and is thermal-labile. Studies of the compound in the ultracentrifuge show it to be polydisperse and to have an increased rate of sedimentation.

A study of the pigment changes during the early stages of gamma irradiation indicates a reaction sequence similar to the addition of hydrogen peroxide, but with some differences. If oxymyoglobin is the starting form of the pigment, the reaction proceeds through the formation of a brown compound, metmyoglobin, then to a red compound whose subsequent behavior depends on the history of the solution. If the red pigment is formed in fresh extracts and the solution removed from the gamma source as soon as it is formed, the spectrum changes to that of oxymyoglobin. Conversely, if aged or previously irradiated solutions are irradiated, the red pigment formed turns brown upon standing. Again, the effect of unknown factors is observed, but—as in the case of sterilizing doses—no additives which duplicate fresh extract conditions have been found as yet.

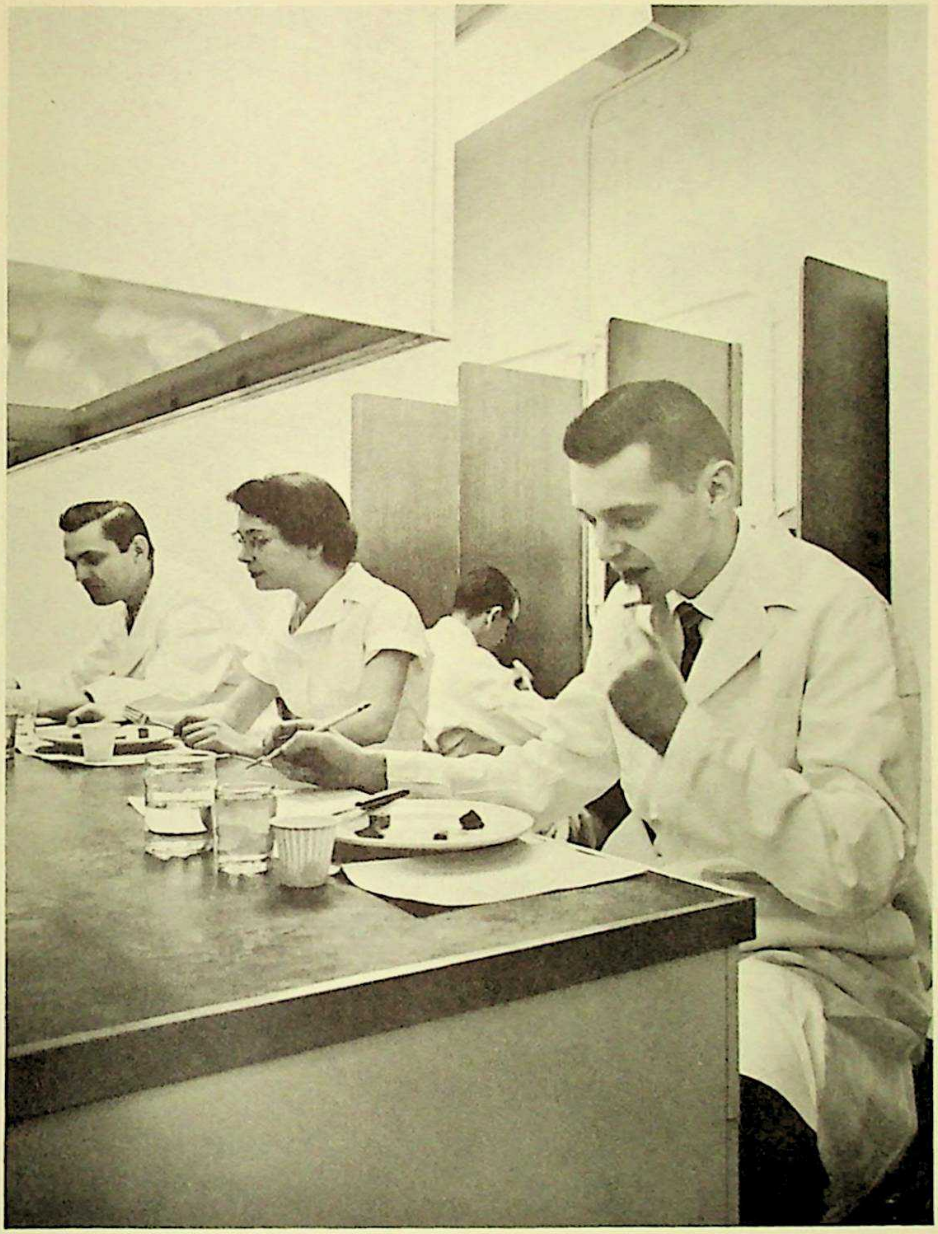
Chemistry of Changes That Occur in Meat Fats during Irradiation

(L. A. Witting and B. S. Schweigert)

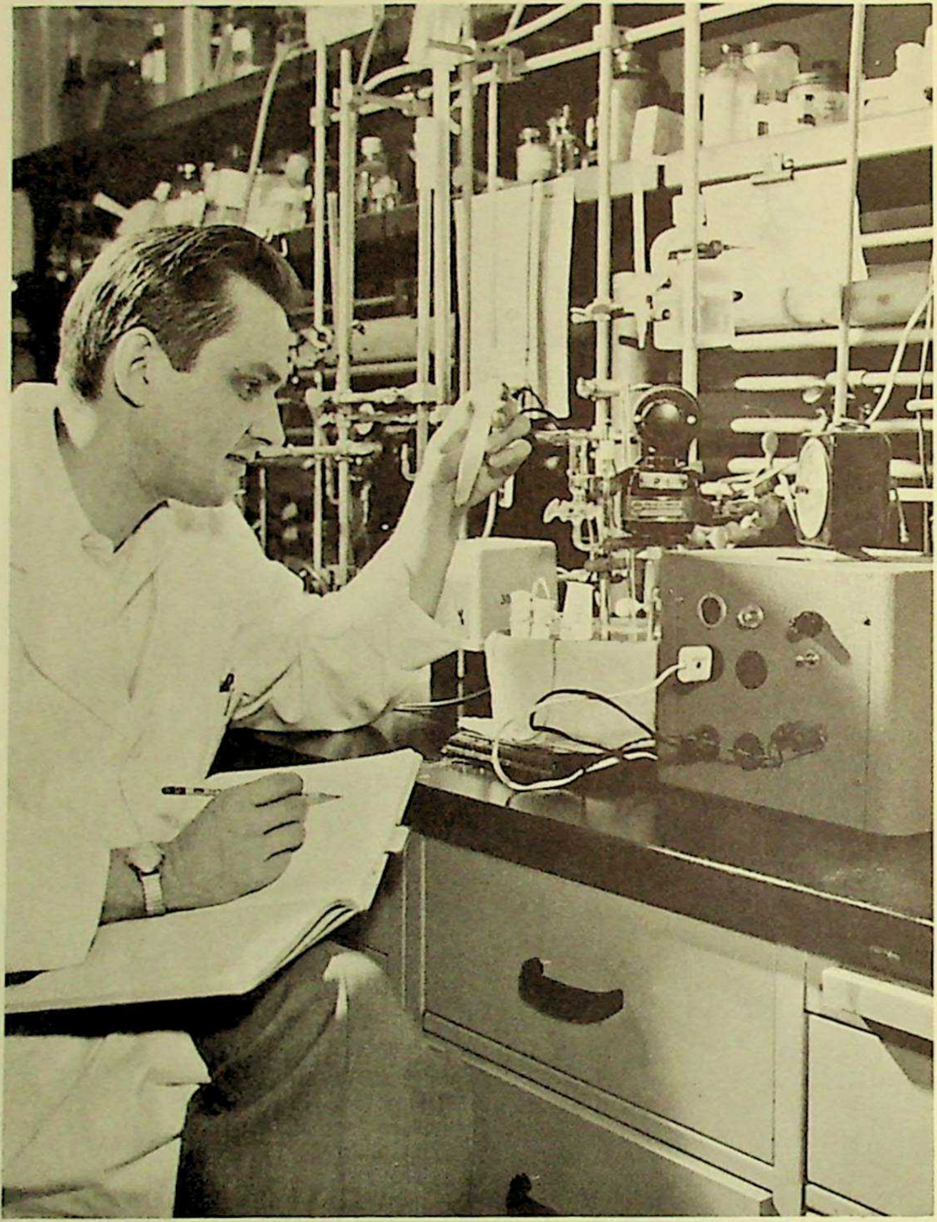
(Supported in part by contract with the Quartermaster
Food and Container Institute for the Armed Forces)

Work has continued on the identification of the 2,4-dinitrophenylhydrazones of the volatile carbonyl compounds, steam-distilled from solvent-extracted hog back-fat irradiated at 10 megarep. The derivatives obtained from 30 pounds of fat were pooled, and duplicate aliquots were placed on replicate chromatographic columns. Slow elution by solvent mixtures of increasing polarity furnished a continuous series of fractions which were analyzed quantitatively, by a spectrophotometric procedure, for saturated and unsaturated carbonyl derivatives. The resulting elution diagram indicated that not less than 24 components were present in the mixture, of which 13 were saturated and 11 were unsaturated carbonyl compounds. A single component, an 11-carbon unsaturated carbonyl compound, accounted for 43.8 per cent mole of the total material. Only two other fractions were present to the extent of greater than 5 mole per cent. Paper chromatography will be used to characterize these derivatives further.

The interaction of sulfur compounds and carbonyl compounds has also been investigated. In this connection, a number of condensation products have been prepared in the laboratory. These have included the condensation of methyl mercaptan with compounds such as acrolein, crotonal, acrylic acid, crotonic acid, and the condensation of isopropyl mercaptan and acetaldehyde. An effort has been made to determine the nature of the oxidation products of methional and related compounds, since these products appear to be related to the odor noted in irradiated meat. In collaboration with the Quartermaster laboratory at Natick, Massachusetts, samples are currently being examined by mass spectrometry in an attempt to detect the unstable intermediates in the oxidation mixture.



Carefully selected and trained AMIF taste panels evaluate results in many research projects. Here panel scores effect of tenderization treatment of beef.



Studies to determine the precise chemistry of meat-color pigments and of changes in the pigments occurring during curing, cooking, and other processes continue. A. J. Siedler here seeks purified extract of cured-meat pigments.

Meat Pigments

Studies on Myoglobin and Related Heme Pigments in Muscle Tissue

(J. B. Fox, A. J. Siedler, C. Bernofsky,
and B. S. Schweigert)

Earlier studies have shown crystalline myoglobin to be separable electrophoretically into three components, approximately 80, 20, and 1 per cent of the original compound. Acid-base titration of the three compounds was undertaken in order to define better the differences between the three, which might be of physiological or chemical importance.

Some titration curves on the major component in myoglobin have been obtained, but it has been found necessary to secure accurate spectral data for the interpretation of the results. Available spectra and extinction coefficients for myoglobin are meager and conflicting. Furthermore, for the spectral analysis of mixtures of more than one form of myoglobin, it is necessary to have extinction coefficients for each component for as many wave lengths as there are components. These data are not available. For this reason, the compilation of spectra of the various forms of myoglobin has been undertaken. A spectrum of metmyoglobin has been obtained, ranging from 230 to 600 $m\mu$, and a partial spectrum for myoglobin. Technical difficulties have been met in obtaining spectra in the ultraviolet region, since most reductants absorb in this region. Special cells have been designed to overcome part of these difficulties.

A technique has been worked out for the preparation of heme-free globin, this colorless protein showing the same physical characteristics in the ultracentrifuge as the native heme-complexed material. This preparation, being free of the interfering heme, is valuable for the study of the physical chemistry of myoglobin, since most of the gross physical properties of the heme-globin complex are dependent on the globin moiety.

Preliminary studies on the nature of the primary cured-meat pigment were initiated, using purified myoglobin preparations in a model system. The sequence of reactions in the formation of the denatured cured-meat pigment was surveyed in this system. Acetone extracts of the pigment obtained from the model system and pigment obtained for dried, cured beef were almost identical.

Attempts to purify the acetone extract of the pigment with a variety of adsorbents have not been successful to date. Some preliminary procedures, using commercially prepared casein as adsorbent, have indicated that the pigment extract may be resolved into two components, presumably an oxidized and a reduced form.

After acetone extraction, the pigment is soluble in diethylether and is quite stable stored in the dark under refrigeration. However, petroleum ether solutions of the pigment are highly unstable. Prolonged washing of a diethylether solution of the pigment also causes rapid decomposition of the pigment to a hemin-type compound.

Robert A. Sliwinski utilizes recording spectrophotometer during studies designed to determine nature and manner of functioning of natural enzymes that tenderize beef during aging.

Enzymes

The Study of Proteolytic Enzymes in Beef-Muscle Tissue

(Robert A. Sliwinski and D. M. Doty)

The first approach to this problem involved developing a sensitive method for determining the over-all proteolytic activity of meat and meat extracts. The use of hemoglobin as a substrate was tried chiefly because of the solubility of hemoglobin at lowered pH's and because of the simplicity of assaying for degradation products as an index of proteolytic activity.

Hemoglobin powder was dissolved, dialyzed for 16 hours against tap water, followed by 24 hours of dialysis against distilled water prior to denaturing with urea. The denatured hemoglobin was then buffered with acetate at a pH of 4.0, the final concentration being 2.0 per cent hemoglobin in 0.2 M acetate buffer.

Beef round steak was used as the source of the enzyme. Only the lean portions were finely ground. The ground beef was homogenized with water in a Waring Blendor, the pH adjusted to whatever pH was to be used for the extraction, the slurry was rehomogenized, and the supernatant collected after centrifugation. The supernatant was adjusted to pH 4.1 immediately prior to incubating with the hemoglobin substrate for 24 hours at 37° C. The final concentration of the hemoglobin substrate in the reaction mixture was 1.0 per cent in 0.1 M buffer. Trichloroacetic acid was added to stop the reaction, and the amount of soluble tyrosine liberated from the substrate was determined spectrophotometrically at a wave length of 276 μ. The amount of tyrosine liberated was then used as an index of proteolytic activity.

Preliminary experiments showed that extraneous materials which absorbed in the range of tyrosine were extracted from the meat along with the enzymes and thereby interfered with the assay. The removal of these unwanted absorbing substances was attempted. It was found that after the meat was extracted, dialysis of the extracts prior to the assay of the enzymes almost completely removed the extraneous absorbing substances without loss of proteolytic activity.

The data from these experiments indicate that proteolytic activity increases after dialysis of the meat extracts prior to the assay, and the activity was greater when the meat was extracted at a higher pH. All extracts were adjusted to a pH of 4.1 prior to running the assay; therefore, all assays were run under identical pH conditions.

Further work is outlined to substantiate these results, along with determining optimal conditions with regard to temperature, incubation time, pH, enzyme substrate concentration, etc.

Water Balance

Water-binding Capacity of Meat

(M. C. Urbin, W. J. Aunan,[*] and G. D. Wilson)

Considerable time has been utilized to review the known information relating to water balance and the determination of water in muscle tissue. From the many approaches which suggested themselves, the decision was made to investigate the distribution of intracellular, extracellular, and interstitial water, both in the live animal and in the carcass, and to attempt to relate the findings to the moisture-retaining properties of muscle.

Techniques for determining extracellular water, blood volume, and total body water have been worked out. Inulin is used for measuring extracellular water, Evans blue for measuring blood volume, and antipyrine for measuring total body water. The total body water minus the extracellular water indicates the intracellular water, and the extracellular water minus the blood water will give the interstitial water. This information will be related to post mortem water distribution, and, when combined with studies of ante mortem and post mortem treatments, it should be possible to establish the relationship between water balance and moisture-holding properties.

Meat Processing

The Effect of Sodium Ascorbate and Sodium Iso-ascorbate on the Quality of Frankfurters

(F. Mills, D. J. Ginsberg, C. Edith Weir, G. D. Wilson)

(Supported in part by grant from Merck and
Company, Inc., Rahway, New Jersey)

Three types of frankfurters, representing products of different quality, were prepared with and without sodium iso-ascorbate added to the emulsion. A shorter processing time was required for frankfurters containing sodium iso-ascorbate to produce a product as acceptable as, or more acceptable than, the control. Acceptability of the product was based on taste-panel scores for tenderness, juiciness, texture, and flavor, a subjective peelability score, and spectrophotometric comparisons of internal color. The results support other work which indicates that ascorbic acid, sodium ascorbate, sodium iso-ascorbate, and possibly sodium iso-ascorbic acid can be used interchangeably on a molar basis in frankfurter production. Suggested processing schedules were developed for the production of frankfurters prepared with and without sodium iso-ascorbate.

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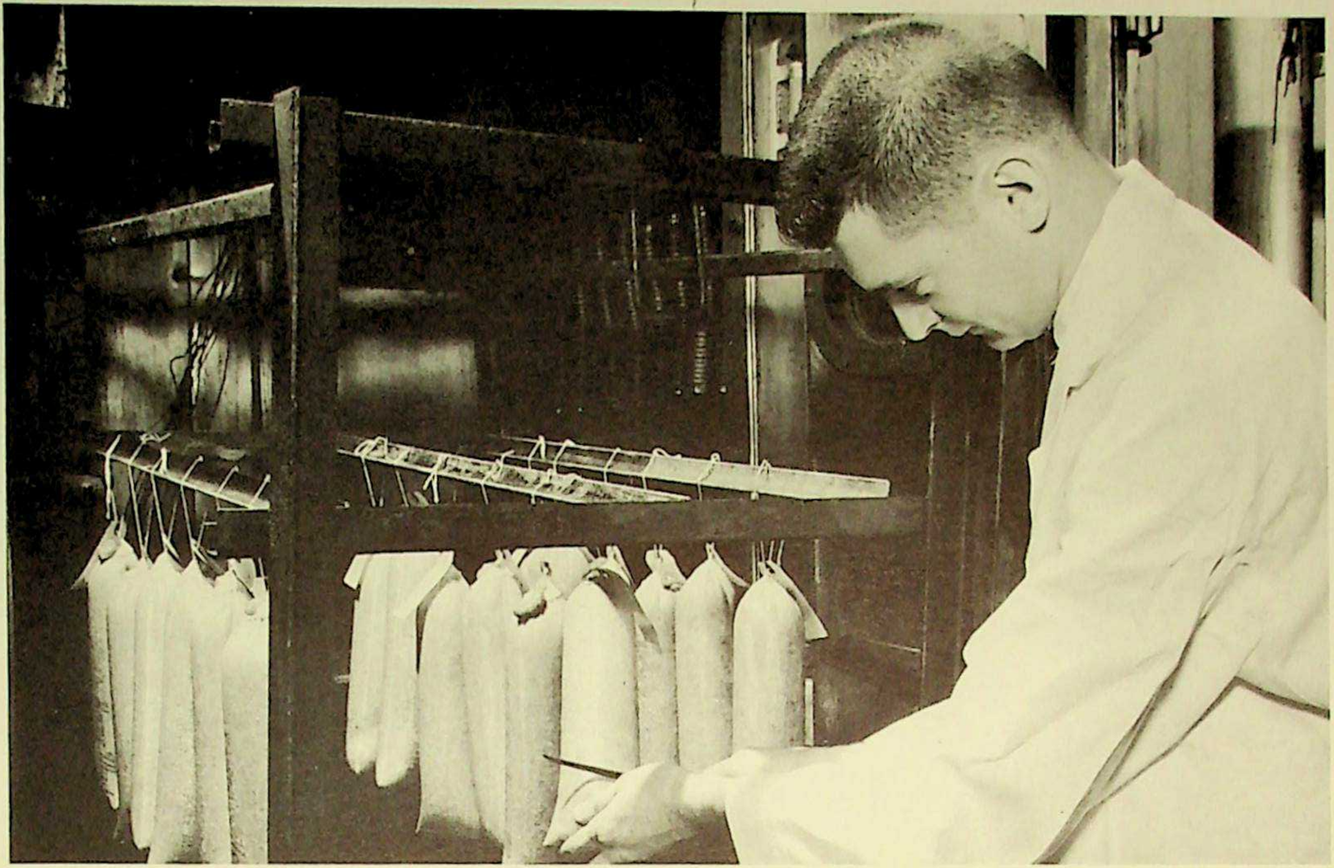
[*] F. C. Vibrans Fellow 1957.

Techniques for determining water in various tissue components constitute first objectives of Dr. M. C. Urbin, of AMIF Food Technology Division, and Dr. W. J. Aunan, of the University of Minnesota, in study of water-binding capacity of meat. Dr. Aunan aided in project during summer studies conducted under provisions of AMIF F. C. Vibrans Fellowship for senior scientists.



Frank Mills, of AMIF Food Technology, inspects pork roll produced in Foundation sausage kitchen as phase of highly successful experiments to provide information assisting in the development and use of starter culture in fermented-type meat products.

A Study of the Bacteriology of Summer Sausage

(R. H. Deibel and C. F. Niven, Jr.)

The problem of producing highly active lyophilized preparations of Pediococcus cerevisiae appears to have been solved. It has been found that lyophilization reduces the salt tolerance of the culture so that it may be unable to grow in an ordinary sausage emulsion. However, routine carrying of the culture in media containing 6.5 per cent salt before lyophilization appears to have eliminated this difficulty.

A patent has been applied for covering the use of P. cerevisiae as a starter culture for fermented sausages, and a non-exclusive license has been granted to Merck and Company, Inc., to produce the lyophilized starter culture for commercial use. The Merck preparation is being marketed under the trade name ACCEL.

A Study of Pediococcus cerevisiae as a Starter Culture for Lebanon Bologna, Pork Roll, and Genoa Salami

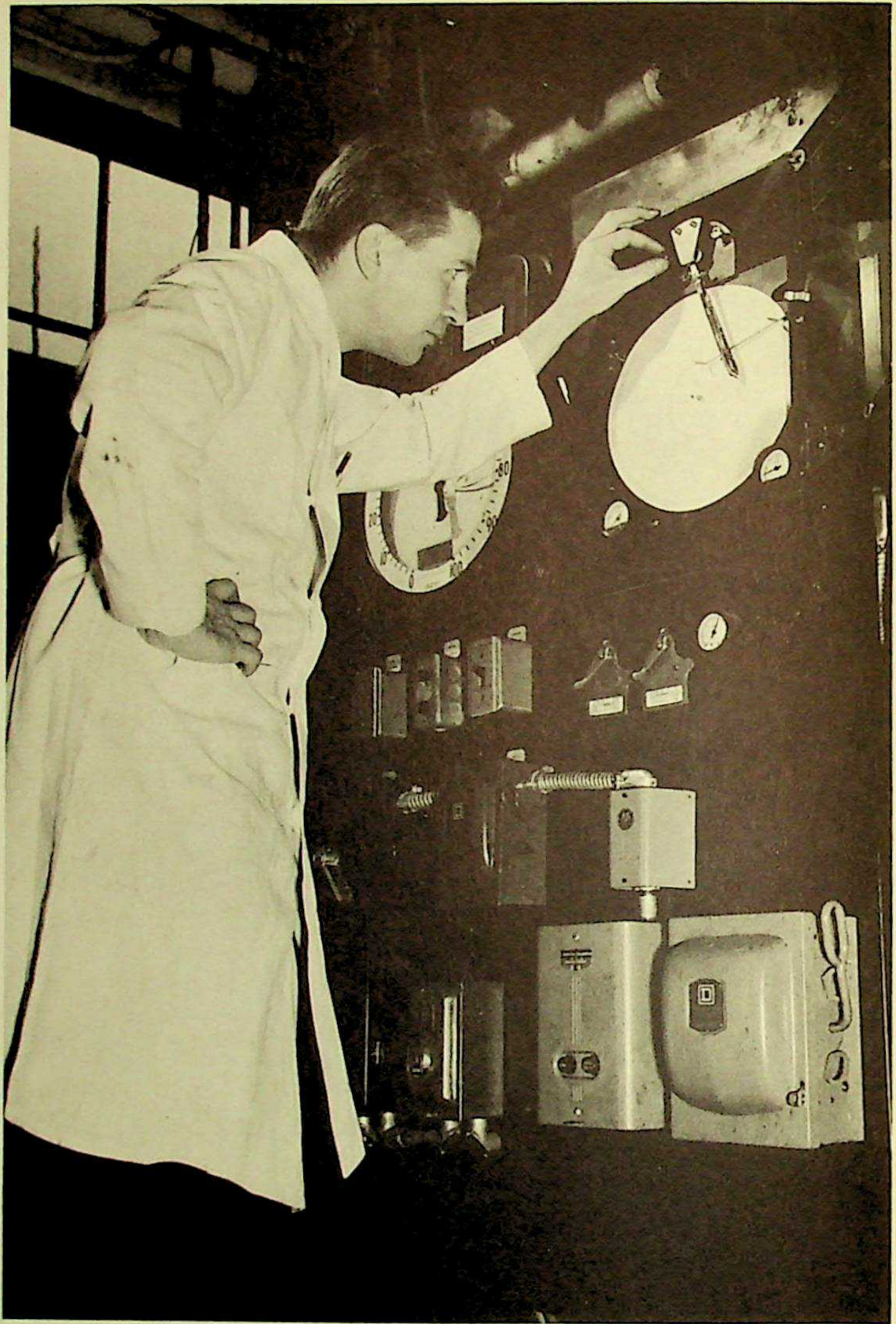
(J. M. Baron, R. H. Deibel, and G. D. Wilson)

(Supported in part by a grant from
Merck and Company, Inc.)

Information gained in the successful development of Pediococcus cerevisiae as a starter culture for Thuringer-type fermented products suggested that such a culture also could be utilized in other types of fermented sausage products.

The work, to date, has been confined to the preparation and study of Lebanon bologna. Experimental variations have been made in the level of starter culture, spice formula, and processing schedules, in an attempt to duplicate commercial samples of the same product. Procedures for the determination of lactic acid have been adapted to follow the rate of acid production in sausage with various levels of inoculum. This information will permit a more accurate control of the amount of acid production in such sausage.

Pilot-plant studies have produced acceptable products with respect to flavor and color, but further study is needed to duplicate more nearly the characteristics of commercial products.



Martti Knuuttila, of Finland, conducting research studies in the Foundation's Division of Food Technology under a Fulbright Fellowship, sets instruments recording smoke density, humidity, and temperature during operation of AMIF experimental smokehouse.

Bacteriology

Microbial Oxidation of Fats

(Richard Berk,* James B. Evans, and C. F. Niven, Jr.)

Nutritional studies have shown that the growth of some micrococci was stimulated by oleic acid. These organisms could oxidize oleic and a variety of other acids but could not use these acids as a sole source of carbon. However, strains of Pseudomonas aeruginosa and Aerobacter aerogenes were found that could oxidize oleate and utilize it as the sole carbon source in a growth medium.

Cell-free extracts of a Pseudomonas have been shown to oxidize oleate virtually to completion (CO_2 and H_2O) and thus do not accumulate any intermediates. These extracts have been fractionated, and attempts have been made to trap carbonyl intermediates, but no incomplete oxidation products have been detected. The complete system appears to be soluble in cell-free extracts, and no cofactor requirements have been demonstrated.

Studies also have been conducted on preparations from an Aerobacter. There was some evidence that some keto acids may accumulate during the oxidation of oleate by these preparations. However, the nature of this compound(s) has not been determined.

Studies are continuing on these fatty-acid oxidation systems and may be extended to include other acids and other micro-organisms.

Studies on Halophilic Bacteria from Salted Meats, Hides, and Casings

(I. S. Schneider† and C. F. Niven, Jr.)

This study has been concerned mainly with the isolation and characterization of a red-pigmented, halophilic micro-organism from Brazilian charque. Charque is a salted and dried meat that is produced and consumed in large volume in Brazil and some other South American countries. One of the greatest spoilage problems during the production of charque is a red discoloration, accompanied by a characteristic offensive odor.

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*W. Lee Lewis Fellow, 1957.

†Dr. Schneider, a citizen of Brazil, has been supported by a fellowship from the Brazilian government.

A halophilic, red-pigmented bacterium has been isolated from some red-discolored charque. This organism appears to be a member of the genus *Pseudomonas*. It is a Gram-negative, somewhat pleomorphic, rod that is *motile by* means of polar flagella. It is aerobic, catalase-positive, digests gelatin, starch, and casein, and does not produce acid from glucose, lactose, or sucrose. It produces H_2S but does not produce indole or reduce nitrate. This organism grows best in media containing 18-25 per cent salt, grows poorly in media containing 16 per cent salt, and does not grow in media containing 14 per cent or less salt.

This organism is only moderately resistant to heat, being killed by 5 minutes at 65° C, but surviving 30 minutes at 60° C. It is resistant to most antibiotics other than bacitracin and is more resistant to gamma irradiation than most non-spore-forming bacteria.

Media have been developed that provide relatively rapid and vigorous growth of this bacterium, and it has been reinoculated onto dried salt beef, salted hides, salted fish, and salted casings. It grows on all these products and produces a red discoloration and the typical offensive odor. Growth was best on the salted fish and poorest on the salted casings.

Work is continuing on the temperature limits of growth, nutrition, and physiology of this micro-organism.

Occurrence and Significance of Enterococci in Hams

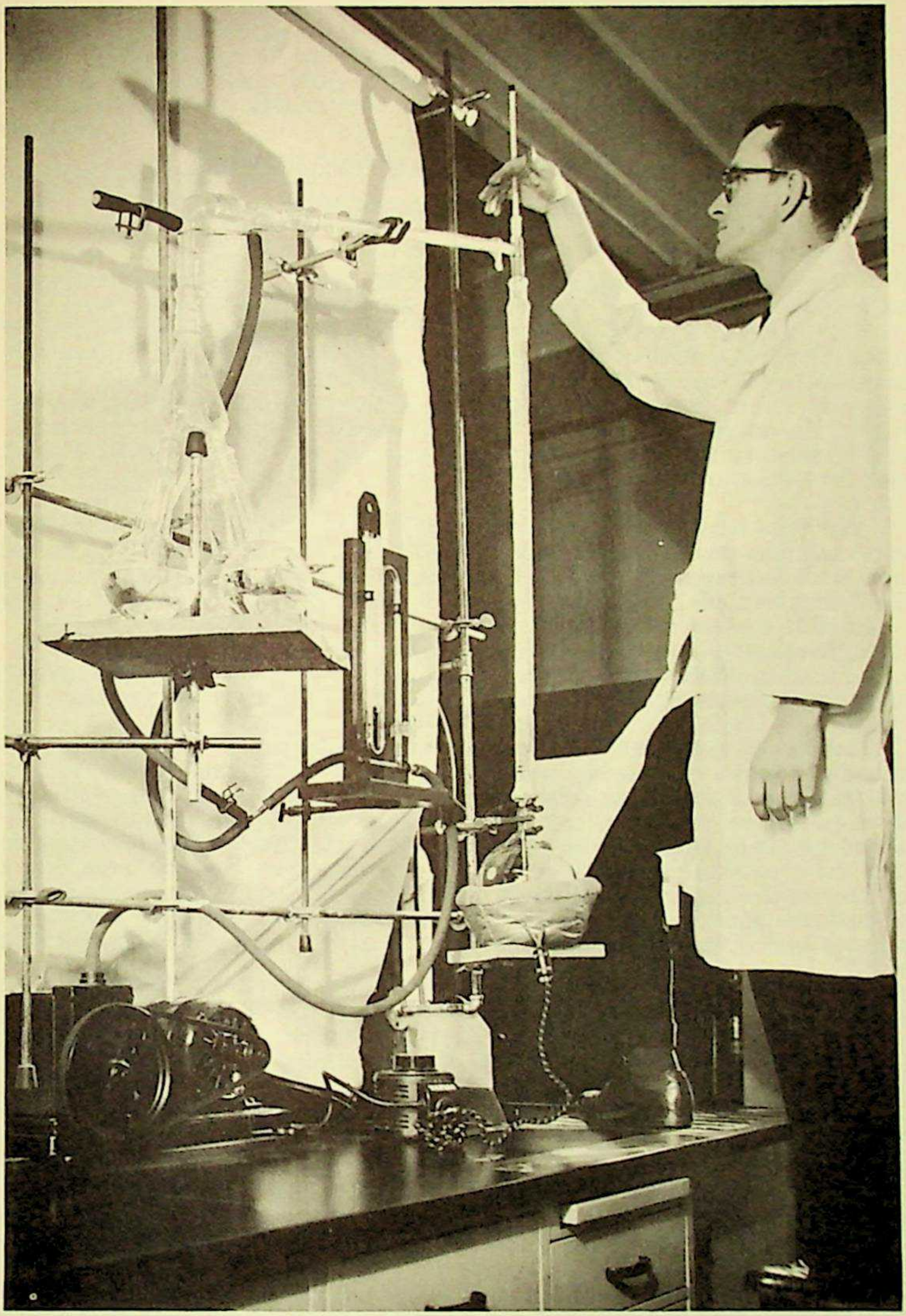
(R. H. Deibel, Donald Lake, and C. F. Niven, Jr.)

Previous work in this laboratory has confirmed the occasional occurrence of *Streptococcus faecium* in canned hams and has added to the taxonomic *differentiation between* this organism and *S. faecalis*. These studies have been extended to include a more *representative collection* of enterococci, including freshly isolated strains from human feces, food products, and botanical plant sources.

The results have confirmed our previous reports that the enterococci may be divided into two main groups: *S. faecalis* and its varieties and the *S. faecium* - *S. durans* group. *Studying ten fecal* samples, *S. faecium was the predominant* enterococcus in eight, and *S. faecalis* was *predominant* in two. This seems to contradict the reports *of Barnes et al*. that *S. faecalis* is of human origin and *S. faecium* of porcine origin.

Through the courtesy of Dr. J. Orvin Mundt, of the University of Tennessee, a large collection of enterococci from botanical sources and food-processing plants has been obtained. In addition to typical *S. faecalis* and *S. faecium*, this collection included quite a number of *strains* that *appear to be* intermediate between these two species, although they may be somewhat closer to *S. faecium*.

Studies on the heat tolerance of strains of *S. faecalis* and *S. faecium* have been conducted.



Hal T. Slover, of AMIF Division of Organic Chemistry, checks temperature of take-off during distillation of by-product residues of extreme fat-processing as he investigates technique for the preparation and measurement of fat-oxidation products.

Animal Fats

A Study of Fat Stability in Chilled Doughs

(C. Edith Weir, Audrey Freeman, and L. R. Dugan)

Pastry doughs containing 44 per cent fat based on the flour content were wrapped in moisture-vapor-proof cellophane bags and stored at -10°, 0°, and 20° F. for periods of 2 - 36 weeks. The following fats were studied: hydrogenated vegetable shortening, unstabilized lard, and lards stabilized by Tenox II, Tenox IV, propyl gallate, Tenox IV + propyl gallate, citric acid, butylated hydroxyanisole, and butylated hydroxytoluene. Part of the dough was extracted with benzene for peroxide and free fatty acid measurement. The remainder of the dough was baked at 425° F. and used for Schaal oven tests.

The free fatty acid content of the pastry was not affected by the storage conditions studied or the type of stabilizer used in the fat. The Schaal oven storage time decreased with the increase in the chilled storage time of the doughs made from each of the fats. This was associated with a rise in peroxide content during the first 12 weeks of storage; after this, the peroxides gradually decreased in amount until they reached their initial concentration. There was very little difference in Schaal oven life or peroxide development among the doughs held at the different temperatures.

The effect of the initial moisture content of baked pastries upon the stability of the fat was tested at elevated storage temperature (63° C.). Pastries were baked from doughs containing 44 and 66 per cent of fat based on the weight of the flour they contained. The fat used was lard stabilized by Tenox II, Tenox IV, and propyl gallate. Distilled water was added to the crushed pastry wafers to raise the moisture content from 5 to 10 per cent at the beginning of Schaal oven storage. The moisture content of the pastries soon equalized, but the Schaal oven life was longer for the samples having the higher moisture content at the beginning of the storage period.

Fundamental Studies in Pork Rancidity

(L. R. Dugan and Arne Jensen)

The project for determination of the causes of pork rancidity has been initiated. Preliminary investigations have involved the preparation of hemoglobin from blood and the preparation of methyl and ethyl esters of hemin.

The methods for analytical determination of heme compounds have been studied, and standard curves for their determination have been prepared. Initial studies will involve a heme catalysis of oxidation of linoleic acid suspensions. Methods have been investigated for preparing heme suspensions at controlled pH. The use of ultrasonic treatment has resulted in the preparation of suitable suspensions with a much better control of pH than is possible through the process beginning with formation of the ammonium soaps. It has been found desirable to prepare the use of solutions under a blanket of inert gas during a period of ultrasonic treatment.

Some investigation has been made of the effect of sodium chloride on the pH of solutions, since this has been cited in the literature as being pertinent to rancidification. It has been found that the chief cause of change in the pH of sodium chloride solution is the absorption of CO_2 from the air and that, when this is excluded, the pH of a sodium chloride solution is 6.9, which is essentially neutral. In considering the effect of heme compounds, it is necessary to determine whether solubility is pertinent. The heme esters, which have been prepared, have been found to be appreciably soluble in linoleic acid.

Uses of Inedible Animal Fats

(Hal T. Slover and L. R. Dugan)

Influence of Gamma Radiation on the Autoxidation of Methyl Oleate: Sites of Oxidation

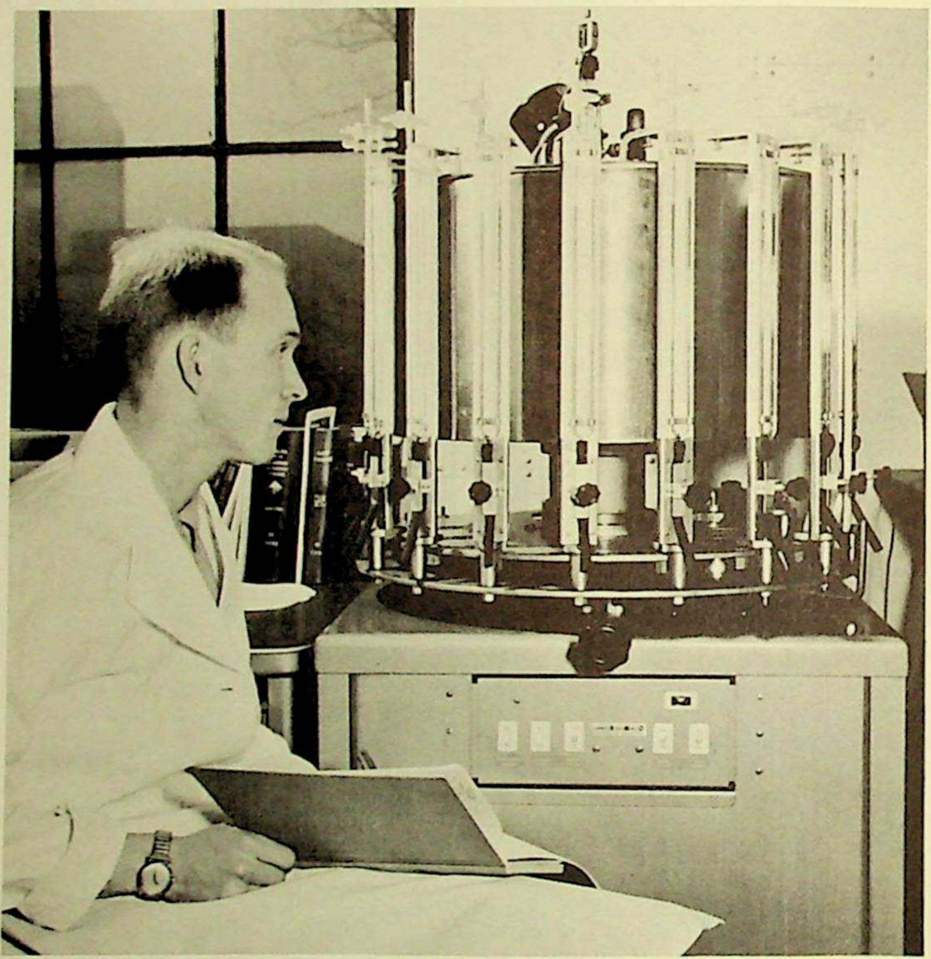
The hydroperoxides formed from methyl oleate under gamma radiation have been shown to be the same as those formed without irradiation. In each case the 8-, 9-, 10-, and 11-hydroperoxide oleates are formed. The effect of gamma radiation is therefore apparently limited to accelerating the autoxidation rate, without activating any new sites not attacked in the absence of radiation. Among the products of the autoxidation were non-anoic, octanoic, non-andioic, and octandioic acids. A sample autoxidized in the presence of cobalt stearate also formed small amounts of what appeared to be heptanoic and hexanoic acids.

Influence of Pressure on the Autoxidation of Methyl Oleate

In an attempt to find additional ways of accelerating autoxidation, samples of methyl oleate were oxidized at various temperatures under oxygen pressures up to 800 psi. These experiments were carried out on small samples distributed on glass wool. At 120°C., under pressures above 600 psi, methyl oleate explodes after about 45 minutes. Samples oxidized for shorter times under these conditions indicate an oxidation rate about four times as fast as that at atmospheric pressure. The acceleration drops off rapidly at lower temperatures and pressures.

Use of Near-Infrared Spectra in the Study of Autoxidized Fats

This study was undertaken in the hope of finding some easier way of analyzing the complex mixture present in autoxidized fats. The oxidation products—hydroperoxides, alcohols, ketones, aldehydes, and acids—all have absorption bands in the narrow range from 2.7 to 3.0 μ. Spectra of known compounds and mixtures in CCl_4 solutions showed that a mixture of these compounds gives three different bands, attributable to: (1) alcohols, (2) hydroperoxides and acids, and (3) ketones and aldehydes. Alcoholic -OH and hydroperoxidic -OH are easily distinguishable in mixtures. This is the first report of a clear-cut resolution of these two bands. Hydroperoxide and carboxylic -OH lie too close together to be resolved and appear as one band. The > c = o absorption (first overtone) of aldehydes and ketones combines with that of the ester carbonyl to give one broad weak band. Spectra of successive samples of autoxidizing methyl oleate clearly showed the increase in each of the bands as the oxidation proceeded. One such series, run at a low temperature to reduce acid formation, gave a straight-line plot of -OOH absorption versus PV, with a slope (d2815/moles peroxides) of 0.35.



Warburg respirometer is utilized by Arne Jensen, of Division of Organic Chemistry, during investigations relating to the determination of cause and possible prevention of rancidity in pork products.

Frozen Foods

The Effect of Freezer Storage upon the Quality of Cooked Pork Chops

(C. Edith Weir and Audrey Freeman)

Pork chops were precooked to 140° F. and 185° F. internal temperature. They were wrapped in LSAT-300 cellophane and stored at 0° F. for 2 - 24 weeks. Raw pork chops from the same loins were wrapped and stored in the same manner. The chops were heated in a broiler at 400° F. atmospheric temperature to an internal temperature of 140° F. before taste-panel judging. Direct comparisons were made between stored raw and precooked chops. The chops, compared to those precooked to 185° F., were also heated to 185° F. rather than 140° F. The chops were compared on the basis of aroma, flavor, juiciness, tenderness, and texture.

The desirability of the aroma of all the chops decreased after more than 10 weeks' storage. Flavor, tenderness, and juiciness scores for the lean portions of the chops were not affected by the storage periods studied. The fat developed a rancid flavor after 16 weeks of freezer storage. Rancidity was apparent in the odor after 20 weeks. Flavor ratings were similar for chops cooked to 140° F. prior to storage and those stored raw. The fresh chops cooked to 185° F. were preferred flavorwise to those precooked to 185° F. Precooked chops were less tender than those cooked from the raw frozen state. Those cooked to 140° F. were more tender than those cooked to 185° F. Those precooked to 185° F. were dry. Those cooked to 140° F. were very juicy or watery. The heating time required to reach 140° F. was the same for raw chops and those precooked to 140° F.; chops precooked to 185° F. required 20 per cent less time to reach 140° F.

These data indicate that precooking pork chops to 140° F. or 185° F. does not affect their flavor quality during 20 weeks of storage at 0° F. Precooking to 140° F. did not effect a shortening of the cooking time when the chops were reheated to this temperature. Precooking to 185° F. resulted in a dry, tough, undesirable product when reheated.

Animal Feeds

Studies on the Energy Content of Animal By-Products and Related Materials

(Marion P. Cullen and O. H. M. Wilder)

Two types of energy measurements are being made. In the first, chickens are being used to measure the metabolizable energy content of fats. In the second, rats are being used to measure the productive energy of feedstuffs, using sucrose as a reference standard. One chick test has been completed, and a second is in process. Two rat tests have been completed.

The metabolizable energy of fats in chick diets is being measured at Cornell University, utilizing a semipurified reference diet. In our studies we are employing a practical-type reference diet. Since there is considerable evidence that energy utilization varies with the presence, absence, or quantities of other dietary constituents, data from these chick tests will be reported at a later date.

In the rat tests, using sucrose as a reference standard, data have been obtained that indicate choice white grease has a higher productive energy content than do any of the so-called hydrolyzed fats or methyl esters of fatty acids. Corn oil was well utilized. It is not generally used in feeds because of its high cost; however, it was employed in these tests as a reference fat.

A Study of the Nutritive Value of Meat Meal and Tankage

(Barbara R. Gregory and O. H. M. Wilder)

This is a continuation of work reported in previous years. Average values for fourteen amino acids have been determined for meat and bone meal, digester tankage, and feather meal. The glycine content of the samples has been determined since last year's annual report. Average values for glycine are: meat and bone meal, 7.19 per cent; tankage, 6.65 per cent; and feather meal, 6.84 per cent. The method for determination of glycine had previously been rather troublesome, but these results are thought to be reasonably accurate. Data on the material content and feeding tests on a series of samples have been summarized and published in a Foundation bulletin.

Taste-panel samples of pork chops are prepared by Audrey Freeman, of AMIF Division of Home Economics, during study of effect of freezer storage on quality of precooked pork chops.

A Study of Inorganic Growth Factors in Animal-Protein Feed Supplement

(Marion P. Cullen and O. H. M. Wilder)

Trace amounts of various minerals are beginning to receive attention as possible growth factors, and requirements for trace minerals are being studied in other laboratories. The ash of various feedstuffs has been shown to stimulate chick growth in certain instances. This study was initiated to see whether the ash of meat and bone meal contained any factor (or factors) that might tend to stimulate chick growth.

A sample of meat and bone meal was divided, and part was fed as is, while the other part was ashed in an electric furnace at 650° C. The ash was then fed to duplicate lots of chicks at such levels as to provide a similar amount to that fed in the positive control meat and bone-meal diet. Another diet was fed in which half of the meat and bone meal plus the ash equivalent to that amount was included. A soybean oil-meal diet was employed as a negative control containing no meat and bone meal. The 8-week chick weights obtained from two tests indicated that the diets containing meat and bone meal or derivatives of meat and bone meal produced heavier chicks than did the soybean oil and control diet. Feed conversion (feed/gain) was improved in the chicks receiving test rations.

The ash of meat and bone meal seemingly contains a factor (or factors) which stimulates chick growth when fed with this type of basal diet. The factor (or factors) has not been identified but should be further studied in relation to such elements as selenium, molybdenum, bromine, etc.

Nutritional Availability of Amino Acids. 1. Lysine

(Barbara R. Gregory and O. H. M. Wilder)

Interest in dinitrophenyl-lysine is based on the supposition that lysine is one of the first amino acids to be damaged if the proteins are overheated in processing and that the E-amino group of lysine might be the point of damage. The microbiological methods for determining lysine are thought to measure total lysine—both intact and heat-damaged, while an animal or bird may be able to utilize only the intact or undamaged lysine molecule.

Measurement of dinitrophenyl-lysine in a number of samples of meat and bone meal has indicated a value averaging about 20 per cent less than the total lysine. In order to test the effect of heat on meat and bone meal, two samples were divided, and one portion was autoclaved for 3 hours at 15 psi (120° C.), while the other portion was heated in a hot-air oven for 3 hours at 120° C. Dinitrophenyl-lysine (converted to lysine) in these samples averaged 78 per cent of the microbiological lysine for the autoclaved portions and 92 per cent for the oven-heated portions.

Another sample of meat and bone meal was divided and portions were (1) left untreated, (2) heated in a retort under 24 psi steam for 2 hours (265° F. or 129.5° C.), and (3) cooked in a dry melter with added fat, for 2 hours at 24 psi (265° F.). All samples were then extracted with Skellysolve, dried and ground. Total lysine and dinitrophenyl-lysine were determined, and attempts are being made to measure lysine by rat growth, using the protein-depleted rat. Analytical results indicate no loss in total methionine, valine, or lysine as determined after acid hydrolysis, but a tendency toward lower values in the retort (steam-pressure)-heated, enzyme-hydrolyzed samples and in dinitrophenyl-lysine was noted. The pepsin digestibility of the three samples remained essentially unchanged.

Improved test procedures are being devised for the determination of the utilization of lysine from these samples by rats fed diets deficient in lysine.

Data on the gross energy content of animal fats, measured with an oxygen-bomb calorimeter, is used by Marion Cullen, of AMIF Animal Feeds, as basis for determination of utilizable energy in chick rations.

Ultra-high speed centrifuge is utilized by Dr. E. P. Marbach during studies leading to development of methods for separation of five different nitrogen-containing fractions from fresh and cured hides as he seeks to determine what changes take place in the chemical composition of hides during curing.

Hides and Skins

Chemical and Physical Properties of the Constituents of Hides and Skins

(J. W. Giffee and G. D. Maier)

The principal concern of the current program is the development of information which can lead to new uses for hides and skins. Since collagen is the major component of skin, it has become the principal target for study. We hope that the knowledge gained will ultimately lead to a means of dispersing collagen reversibly in such a way that its unique molecular properties may be used to practical advantage.

Collagen or collagens have repeatedly been demonstrated to be associated with carbohydrate material. The fact that exhaustive extraction failed to remove all of this carbohydrate suggests that it may be covalently linked and thus be related to macromolecular organization. Current studies are designed to investigate the carbohydrate-protein relationship in skin collagen.

Physical and Chemical Changes Produced in Hides and Skins

(F. L. DeBeukelaer and E. P. Marbach)

In a previous report it was indicated that methods had been developed for the separation of five different nitrogen-containing fractions from fresh and cured hides. Preliminary measurements reported at that time suggested that green salting did not effect a significant loss in nonprotein nitrogen (NPN), while brine curing apparently removed approximately five-sixths of this low-molecular-weight fraction. An extension of this study during the past year permitted application of the procedures to the study of hides that had been commercially cured by brining and green salting. This study revealed the following:

1. Brining effected a net removal of only about one-seventh of the NPN rather than the five-sixths indicated in the earlier report. The salt-soluble or globular fraction was also only slightly reduced; only a little more than one-fifth of the material was removed by the cure.
2. Green salting resulted in no "net reduction" in NPN and removal of only one-sixth of the globular material. (The term "net reduction" is used here to imply that some of the NPN is probably removed but that a compensating amount is formed through proteolytic breakdown during the curing process. It is necessary to

recognize here that the curing process is dynamic rather than static, particularly with regard to the identity of soluble, nitrogen-containing species.)

3. Both methods of cure gave significantly increased yields of nitrogenous material soluble in half-saturated lime as well as increases in the keratin-elastin fractions. It is possible that the surprising increases in lime-soluble material may be the result of denaturation during the salt cures. The lime solutions would be sufficiently alkaline to dissolve such denatured proteins. Variations in the keratin-elastin fractions are not unexpected, since it is difficult to shave the hide pieces uniformly where the cures produce variations in the texture of the grain surface.

Finally, it is pointed out that the purposes of brining are presumably:

1. To preserve the hide
2. To remove non-leather-making proteins
3. To remove water

It is suggested, in view of the above findings concerning nitrogen distribution, that some of the newer preservatives may well offer advantages over salting if applied to washed hides or to hides that have been fleshed, trimmed, and washed.

Equipment Design

Catalytic Oxidation of Dry-Renderer Exhaust

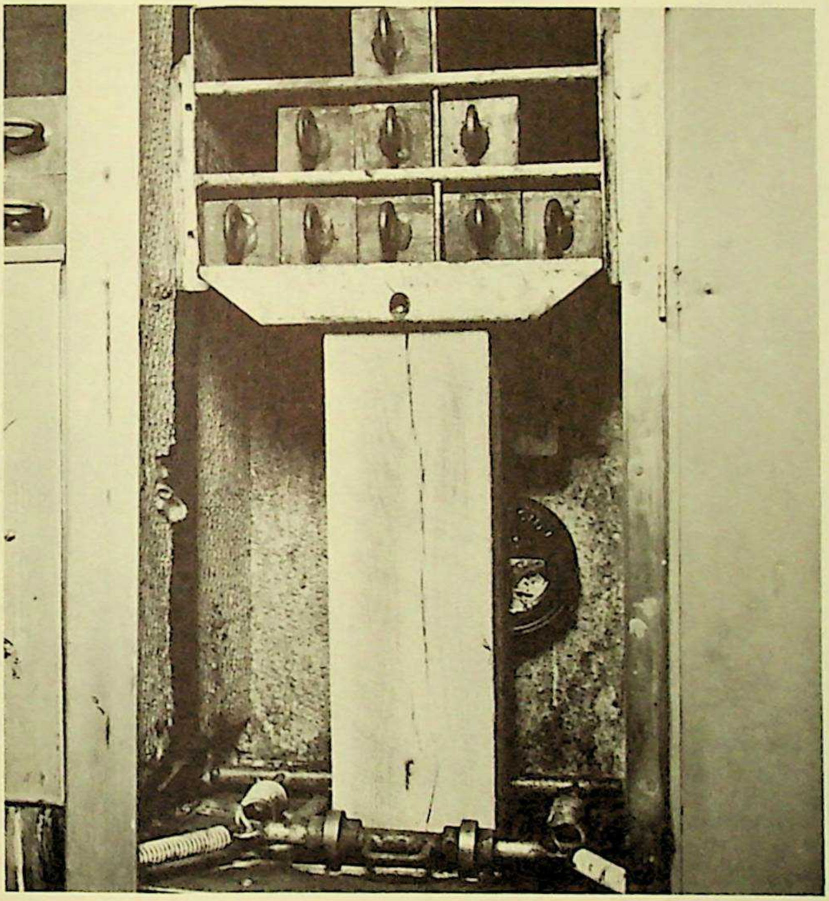
(L. J. Pircon, O. H. M. Wilder, and Richard Harper)

Using a 100-pound-capacity pilot-model renderer with tubular electric heaters in a steel pipe and a platinum-palladium catalyst, all objectionable odors were removed from the renderer exhaust at temperatures of 700°-800°F. In a series of runs this was true for exhaust-vapor flow rates as high as 107 pounds/hour, which required about 90 per cent of the heater output. Data with this pilot-scale renderer indicated that about 50 per cent of the total moisture vapor was driven off during the first third of the rendering run. Any heating unit designed for the combustion of undesirable vapors from commercial renderers would have to be of sufficient capacity to heat the vapors to the required temperature during this peak-load period. Using the data obtained in these experiments, it is possible to calculate the fuel savings that would be expected by using catalytic combustion of undesirable vapors as compared to combustion at higher temperatures without a catalyst.

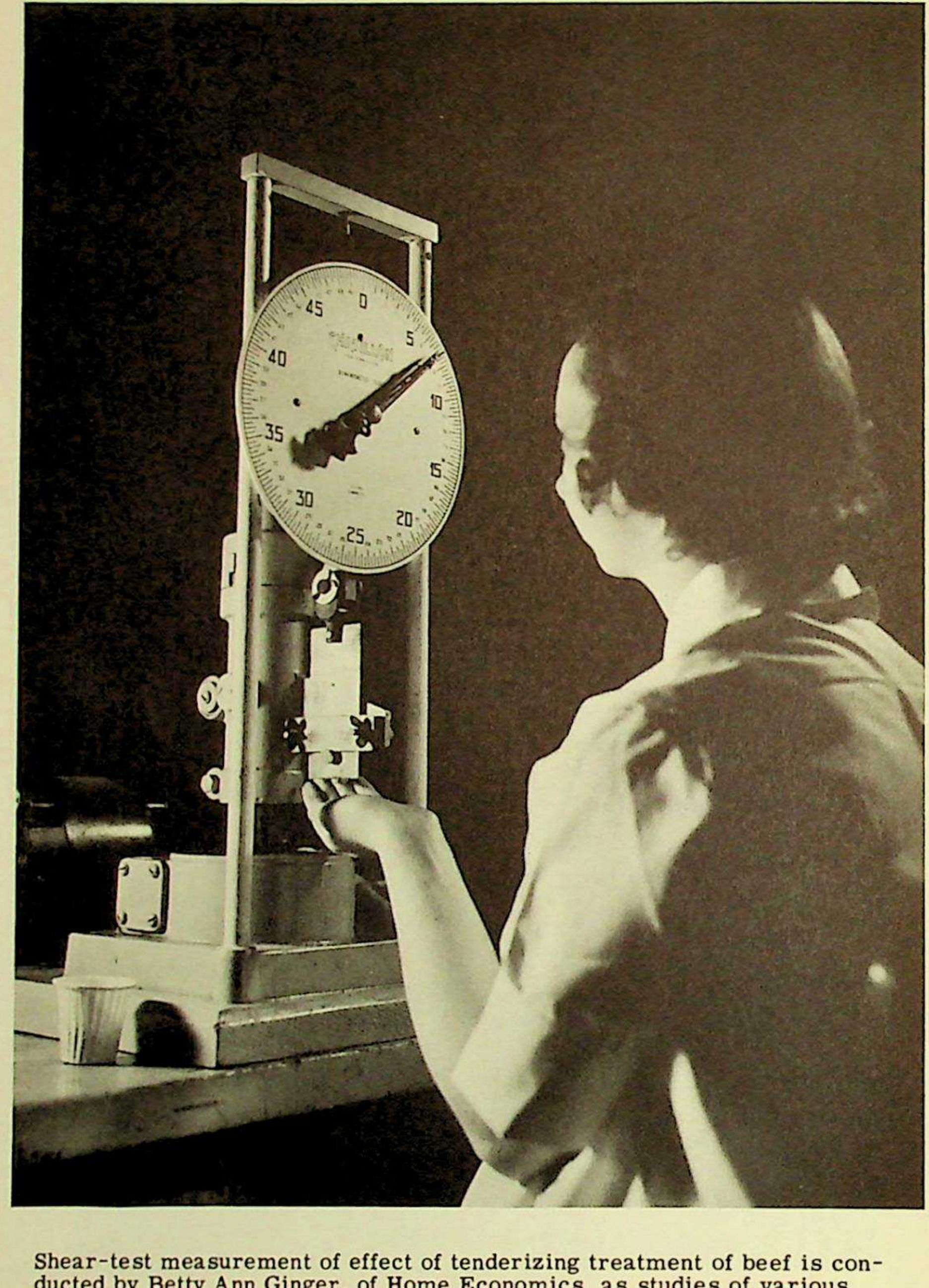
The Design and Development of a Smoke Generator and Density Indicator and Controller

(L. J. Pircon and Richard Harper)

Studies were continued on the frictional wood-burning smoke generator developed by the L. C. Spiehs Company, Inc. Experiments have shown that the amount of smoke generated by the equipment varies directly with the force applied and the consumption rate of the log. Furthermore, the temperature at the interface of the log and friction disk varied with the force applied. In other words, the temperature at the interface under conditions of high smoke production was higher than under conditions of low smoke production. Also, the rate of smoke production under a given vertical force was greatly influenced by the species of the log being burned (oak, hickory, cherry, etc.).



Manner of application of pressure, by weights, to hardwood log in frictional wood-burning smoke generator is demonstrated by photo of interior of generator cabinet.



Shear-test measurement of effect of tenderizing treatment of beef is conducted by Betty Ann Ginger, of Home Economics, as studies of various methods of tenderization continue at Foundation.

IN RECOGNITION

of the interest demonstrated in the application of science for the development of information leading to technological advances in the processing and utilization of products derived from livestock

and in appreciation of financial support accorded to scientific and technological research in those fields which are conducted by the

AMERICAN MEAT INSTITUTE FOUNDATION

at The University of Chicago

The Foundation's Certificate of Membership
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General Meat Co.
Genoa Packing Company
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Albert F. Goetze, Inc.
Gold Medal Packing Corporation
Greensboro Packing Co., Inc.
The Greenwood Packing Plant
Griffith Laboratories, Inc.
Grote & Weigel, Inc.
Gwaltney Incorporated
Chas. Haag, Inc.
Haas-Davis Packing Company, Inc.
Edward Hahn Packing Co.
Handschumacher & Co., Inc.
Harvin Packing Co., Inc.
Heinz's Riverside Abattoir, Inc.
Henry Packing Company
Herrud & Company
Hervitz Packing Co.
Ray Herziger Sausage Co.
High Grade Packing Co., Inc.
Hilo Meat Cooperative, Limited
  Hilo, Hawaii
Chas. Hollenbach, Inc.
Geo. Hollenbach
The Home Packing Company
Geo. A. Hormel & Co.
Hunter Packing Company
Hygrade Food Products Corporation
Illinois Meat Company
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Frank Jaworski Sausage Co.
W. S. Johnson & Co.
Jesse Jones Sausage Company
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Kern County Land Company
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E. A. Kohl Packing Co., Inc.
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Noble's Independent Meat Co.
Noehre Sausage Co.
North Side Packing Co.

Ohse Meat Products Co.
Orleans Canning Co.
Peet Packing Co.
Pegwill Packing Co.
Penobscot Beef & Provision Company
Peters Meat Products, Inc.
Peters Sausage Co.
Scott Petersen & Company
Chas. Pfizer & Co., Inc.
Pryor Packing Co.
Pueblo Packing Co., Inc.
Ralph Packing Company, Inc.
H. J. Rammer Sons
Rapides Packing Company
The Rath Packing Company
P. F. Rathjens & Sons
Redfern Sausage Company
Reimer Meat Products, Inc.
Reliable Packing Co.
Republic Food Products Co.
Roberts Packing Company
Roberts "Turkey Brand" Corned Meats
Rose Packing Company, Inc.
J. H. Routh Packing Company
Rueckert Meat Co.
Russ Meat Co.
Max Russer, Inc.
Saratoga Meat Products Co.
The Wm. Schluderberg-T. J. Kurdle Company
The Schmidt Provision Co.
Schrader's Meat Products
Schweigert Meat Co., Inc.
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Scioto Provision Co.
Seiler's, Inc.
Jos. Seiler & Sons Co.
Sierra Meat Company
Sigman Meat Company, Inc.
Sinai Kosher Sausage Corporation
Smith Packing Co.
Slotkowski Sausage Co.
Spencer, Incorporated
Stadler Packing Co., Inc.
Stahl-Meyer, Inc.
Stanley Provision Co., Inc.
Stark, Wetzel & Co., Inc.
B. D. Stearns, Inc.
Stegner Food Products Co.
Stephen's Meat Products
The Sugardale Provision Co.
Superior Meat Products Inc.
Swift & Company
A. Szelagowski & Son
Table Supply Meat Company
Tee-Pak, Inc.
The Teeters Packing Co.
Tobin Packing Co., Inc.
Trophy Meat Products, Inc.
Wm. Underwood Co.
United Home Dressed Meat Company
United Packing Company
Fred Usinger, Inc.
Valleydale Packers, Inc.
Valleydale Packers, Inc., of Bristol
Valley Pride Packing Co., Inc.
Vienna Sausage Mfg. Co., Inc.
Virginia Packing Company
The Visking Company
Wallerstein Company, Inc.
Wardrup Provision Company, Inc.
Frank Wardynski & Sons, Inc.
Weaver's Famous Lebanon Bologna, Inc.
Weil Packing Co.
Weiland Packing Company, Inc.
Weimer Packing Company
Weisel & Co.
Chas. Wetterling & Sons, Inc.
Robert Wilson & Sons (Food Industries) Ltd., Ayrshire, Scotland
Wilson & Co., Inc.
Winchester Packing Co.
Wisconsin Meat Products, Inc.
Harry Ziemer Co.
Zwanenberg's Fabrieken, N.V., Oss, Holland
Zweigle's

Publications

1. Annual Report. An Outline of Research during the Fiscal Year 1954-1955. American Meat Institute Foundation Bull. No. 29 (1956).

2. A Summary of the Nutrient Content of Meat. B. S. Schweigert and Barbara J. Payne. American Meat Institute Foundation Bull. No. 30 (1956).

3. 7 Steps to Controlled Production of Tangy Summer Sausage in Only 48 Hours. American Meat Institute Foundation Circ. No. 22 (1956).

4. High Levels of Meat and Bone Meal in Broiler Diets. O. H. M. Wilder. American Meat Institute Foundation Circ. No. 23 (1956).

5. End-Point Is Critical Point! American Meat Institute Foundation Circ. No. 24 (1956).

6. Scientific Evaluation of Factors Associated with Beef Quality. D. M. Doty. Annual Meeting, American Meat Institute (1956). (Multilithed.)

7. Science Points the Way to Quality Retention in Fresh and Cured Meats. James B. Evans. Annual Meeting, American Meat Institute (1956). (Multilithed.)

8. Animal Blood. H. R. Kraybill. Encyclopedia of Chem. Tech. 2 (1948).

9. Studies of the Nitrogenous Components of Hides and Skins. I. The Influence of Initial Processing on Freshly Flayed Cattle Hides. F. L. DeBeukelaer and E. P. Marbach. J. Amer. Leather Chem. Assoc. 51, 592-599 (1956).

10. Food Irradiation: Sulfides Released from Gamma-irradiated Meat as Estimated by Condensation with N,N-Dimethyl-p-phenylenediamine. E. P. Marbach and D. M. Doty. Agr. and Food Chem. 4, 881-884 (1956).

11. Tocopherols as Carry-through Antioxidants. L. R. Dugan, Jr., and H. R. Kraybill. J. Amer. Oil Chem. Soc. 33, 527-528 (1956).

12. Animal By-Products Help in Producing High Efficiency Feeds. O. H. M. Wilder. Amer. Feed & Grain (December, 1956).

13. The Sarcolemma and Fibrous Envelope of Striated Muscles in Beef. H. Wang. Exper. Cell Research 11, 452-463 (1956).

14. Feed Stabilization: Vitamin A and Carotene Stability in Feeds Containing Antioxidant-treated Animal Fats. A. J. Siedler, Erica Enzer, and B. S. Schweigert. Agr. and Food Chem. 4, 1023-1029 (1956).

15. Irradiation Effects on Beef: Chromatographic Separation of a Porphyrin Produced from Myoglobin by Gamma-Irradiation. Irene D. Ginger and B. S. Schweigert. Agr. and Food Chem. 4, 885 (1956).

16. Dietary Fats and Atherosclerosis. B. S. Schweigert. American Meat Institute Foundation Special Report No. 1 (1957).

17. Ionizing Radiation in Processing of Meat and Dairy Products. C. F. Niven, Jr. Proceedings of the Conference on Radioactive Isotopes in Agriculture, Michigan State University, East Lansing, Michigan, pp. 265-268 (1956).

18. Effect of Curing Ingredients and Procedures on the Survival and Growth of Staphylococci in and on Cured Meats. R. V. Lechowich, James B. Evans, and C. F. Niven, Jr. Appl. Microbiol. 4, 360-363 (1956).

19. Applications of Physics in the Meat Industry. D. M. Doty. American Meat Institute Foundation Circ. No. 25 (1956).

20. Quality in Sausage Production. C. F. Niven, Jr. American Meat Institute Foundation Circ. No. 26 (1957).

21. Fats in Laying Feeds. O. H. M. Wilder. American Meat Institute Foundation Circ. No. 27 (1957).

22. Discoloration of Sausage: Greening Problems. C. F. Niven, Jr. American Meat Institute Foundation Circ. No. 28 (1957).

23. Bacterial Food Poisoning. James B. Evans, L. G. Buettner, and C. F. Niven, Jr. American Meat Institute Foundation Circ. No. 29 (1957).

24. Prepackaged Meat Products: Slime and Mold Problems. James B. Evans and C. F. Niven, Jr. American Meat Institute Foundation Circ. No. 30 (1957).

25. Problems in Processing: Vinegar Pickled Meats. C. F. Niven, Jr. American Meat Institute Foundation Circ. No. 31 (1957).

26. Dehairing of Hogs. Frank L. DeBeukelaer and Hsi Wang. American Meat Institute Foundation Circ. No. 32 (1957).

27. Complementary Action of Antibiotics and Irradiation in the Preservation of Fresh Meats. C. F. Niven, Jr., and W. R. Chesbro. Antibiotics Annual, 1956-1957, Medical Encyclopedia, Inc. (1957).

28. Ionizing Radiation in Processing Meat and Dairy Products. C. F. Niven, Jr. Atomic Energy Commission Report No. TID-7512 (1956).

29. Annual Report: An Outline of Research during the Fiscal Year 1955-1956. American Meat Institute Foundation Bull. No. 31 (1957).

30. Continuous Thermatronic Processing Cell. L. J. Pircon. U.S. Patent 2,783,350 (February 26, 1957).

31. Role of Vitamin B_{12} in Nucleic Acid Metabolism. V. Liver Deoxyriboaldolase Activity. W. T. Wong and B. S. Schweigert. Proc. Soc. Exper. Biol. and Med. 94, 455-456 (1957).

32. The Foundation . . . and What It Means to You. (1957).

33. Producing Good Quality Fat for Feed Use. O. H. M. Wilder. American Meat Institute Foundation Circ. No. 33 (1957).

34. Means for Sensing and Recording the Temperature on the Contents

of Lard Renderers and the Like. L. J. Pircon. U.S. Patent 2,795,139 (June 11, 1957).

35. Relation of Methional to the Odor of Irradiated Meat. L. A. Witting and O. F. Batzer. Food Research 22, 237-238 (1957).

36. The Stability of Depot Fat from Broilers Fed Rations Containing Animal Fats Treated with Various Antioxidants. A. J. Siedler, Sheldon Moline, B. S. Schweigert, and R. W. Riemenschneider. Poultry Science 36, 449-450 (1957).

37. Studies on Improvements in the Medium for *Lactobacillus acidophilus* in the Assay for Deoxyribonucleic Acid. A. J. Siedler, F. A. Nayder, and B. S. Schweigert. J. Bact. 73, 670-675 (1957).

38. Status of the Use of Animal Fats in Feeds. O. H. M. Wilder, A. J. Siedler, and B. S. Schweigert. American Meat Institute Foundation Circ. No. 34 (1957).

39. AMIF Research—Four Symposia: Meat Processing, Nutrition, Beef Tenderization, and Irradiation. American Meat Institute Foundation Circ. No. 35 (1957).

40. Influence of High Energy Radiation on the Oxidation of Oleic Acid and Methyl Oleate. II. Sites of Oxygen Attack. Hal T. Slover and L. R. Dugan, Jr. J. Amer. Oil Chem. Soc. 34, 333-335 (1957).

41. Product Temperature Measurement in Lard Rendering. L. J. Pircon, C. Francik, E. Golbeck, and D. M. Doty. American Meat Institute Foundation Bull. No. 33 (1957).

42. The Occurrence of *Leuconostoc mesenteroides* in Potato Tubers and Garlic Cloves. M. A. Smith and C. F. Niven, Jr. Appl. Microbiol. 5, 154-155 (1957).

43. The Influence of Enzyme Tenderizers on the Structure and Tenderness of Beef. H. Wang, C. Edith Weir, Marion Birkner, and Betty Ginger. Proceedings of the Ninth Research Conference, Council on Research, American Meat Institute (1957).

44. Simple Design "Twists" Improve Cook Tank. L. J. Pircon. Food Engineering, April, 1957.

45. Fatty Acid Composition of Food Fats and Oils. L. R. Dugan, Jr. American Meat Institute Foundation Circ. No. 36 (1957).

46. Foundation Research on Beef Tenderization. American Meat Institute Foundation Special Report No. 2 (1957).

47. Are Radical Changes in Hog Production Methods in the Making? O. H. M. Wilder. American Meat Institute Foundation Special Report No. 3 (1957).

48. What's This about Controlling "Tang" and Quality in Summer Sausage and Other Fermented Products? American Meat Institute Foundation Special Report No. 4 (1957).

49. Scope and Extent of Research Support by the Meat Industry. B. S. Schweigert. American Meat Institute Foundation Special Report No. 5 (1957).